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Histopathology of liver and kidneys for the assessment of genotoxicity in Indian major carps treated with domestic waste and industrial effluents of Chakbandi drain water

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Abstract

This project was planned to study the exposure effects of domestic waste and industrial effluents on water quality parameters and fish under laboratory conditions. Faisalabad the so called "Manchester" of Pakistan has plastic, leather, textile, dying, printing, finishing, seizing industries, sugar mills and a large number of factories that release the variety of industrial discharges and municipal wastes into River Chenab through Chakbandi drain. In this study, Chakbandi drain's water was collected from the selected sites in the month of April, May and June, 2016 and applied to fingerling's of three Indian major carps i.e. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* under laboratory conditions in glass aquaria. After determining the LC_{50}, the sub-lethal dilutions i.e. 20%, 25%, 30%, 35% and 40% of drain water were tested for three month's acute toxicity trial. After three months, fish tissues i.e. liver and kidneys were taken by dissecting the control and experimental fishes for histopathological studies. Histopathological analysis of control fish revealed the normal structures whereas treated fish showed different abnormalities with respect to liver and kidney tissues. Conclusively, histopathological biomarkers approach was found to be reliable for the assessment of environmental pollution. Moreover, findings of this study are helpful for the evaluation of pollution levels reaching in aquatic fauna particularly fish and indirectly to human populations.

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Introduction

Now a days, aquatic pollution has become the burning issue and alarming problem in Pakistan as domestic sewage and industrial contaminants which contain bulk of toxic chemical compounds, including especially the heavy metals that are constantly discharged into aquatic environments. These heavy metals have drastically toxic effects on aquatic organisms carps (Javed, 2005; Hayat *et al.*, 2007) causes the reduction of haemoglobin that effect the oxygen binding capacity in these aquatic organisms (Ruane *et al.*, 1999; Adeyemo, 2005).

So, there is great need to monitor the toxicity of heavy metals on edible species in aquatic environment. It will enlighten the warning signs of temporal and spatial levels of these processes and also the evaluation of possible effects of metals on human health (Fernandes *et al.*, 2007). Introduction of toxic domestic wastes into water bodies cause decline in biological oxygen demand to the lethal level by decreasing oxygen from the water bodies. Industrial waste water released highly toxic chemicals into rivers, streams and lakes which includes compounds of zinc, lead, copper, mercury and chromium etc. that causing the death of aquatic life at very low concentrations (Shaheen & Jabeen, 2015; Sultana *et al.*, 2016; Zaqoot *et al.*, 2017). Modern agricultural practices are among the important sources for the introduction of pesticides, insecticides and chemosterilants in the water bodies (Sabullah *et al.*, 2015).

Fish can serve as an excellent model organism for the assessment of genotoxicity in water bodies under both laboratory and natural conditions. Since, they are the vertebrates that can metabolize, concentrate and store the genotoxins. Genotoxic evaluation of aquatic environment is the basic mechanism for translating the principle of sustainable development into action. Genotoxic pollutants are found to be associated with gene mutation (mutagenic) and proliferation of tissue (carcinogenic potential). These chemicals can be induced into next generations if unchecked due to their potential damage to cause genetic abnormalities. Though fish die first, next is human. Genotoxicity is deleterious actions that affect the cell's genetic material by influencing its integrity (WHO, 2013).

Histopathological lesions were the primary tools to examine the acute and long term effects of different chemicals in different environmental conditions on living organisms especially in fish (Reddy & Rawat, 2013). Higher level of heavy metals in waste water leading to detrimental histopathological changes vital tissues of fish species due to their tendency of accumulation (Sultana *et al.*, 2016; Gupta *et al.*, 2017) causing the regeneration of reactive oxygen and ultimately leading to severe DNA damage. Therefore, heavy metals are created hazardous effects to aquatic fauna which ultimately lead to serious consequences on human health (Anim *et al.*, 2011; Mir *et al.*, 2014). Hence, there is a need for the utilization of biological monitoring techniques for the analyses of water pollution based on fish with rapid response even on low concentrations of toxicants (Ali *et al.*, 2008; Viana *et al.*, 2013).

Domestic and municipal wastes and Industrial effluents of Faisalabad district are discharged into River Chenab from different directions through a drainage system especially the zigzag Chakbandi drain. Contaminated water includes huge number of chemical compounds from different types of industries including chemical, textiles, soap, pharmaceutical, tanneries, leather and sugar mills etc. which are sufficient to alter the water quality parameters of River Chenab. This devastating alteration in water quality parameters is the main reason for eradication of the aquatic populations of many peculiar and native fish species including Indian major carps i.e. *Catla catla, Labeo rohita* and *Cirrhinus mirgala* etc. This study focuses on the use of biomarker approach to facilitate a base line for fish health evaluation, sustainability and management planning that include the histological changes of gills, liver, kidney and muscles (cellular abnormalities) to visualize the fish health status (Sultana *et al.*, 2016; El-Bassir *et al.*, 2017).

Therefore, histopathological evaluation of fish kidney, liver, gills and muscle tissues are another important segment of methodology to demonstrate frequencies of pathological alterations in fish organs after exposure of municipal and industrial wastewater (Thangam, 2014; Junianto *et al.*, 2017).

Fingerlings of different selected fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* were exposed to five sub lethal survival dilutions of municipal and industrial waste water in laboratory conditions. These Indian major carps are widely cultured in the farms and even harvested from the Rivers of the province Punjab.

Materials and methods

Experimental model

The study was carried out to assess the genotoxicity in Indian major carps through histopathological biomarker approach. The fish fingerlings (11-13g) of three fish species i.e., *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* were procured alive from Government Fish Seed Hatchery, Satiana Road, Faisalabad and shifted in plastic bags containing fresh water filled with oxygen to Fisheries Research Laboratory, Department of Zoology, Government College University, Faisalabad. The fingerlings of fish were bathed for 2-3 minutes in 0.1% $KMNO_4$ to sterilize before acclimatization.

Acclimatization and maintenance

The process of acclimatization of fish was carried out in Fisheries Research Laboratory for two weeks in glass aquaria of 30cm x 60cm x 44.5cm in size filled with dechlorinated and aerated tap water before the start of experiment. All the experimental glass aquaria were washed thoroughly with tap water before use.

Twenty fish of each fish species were kept in each glass aquaria for acclimatization. The fish fingerlings were fed the commercial artificial feed @ 3-4% of wet body weight during the acclimatization period. Proper aeration was supplied continuously to all the glass aquaria with electric air pump (China). The water in the glass aquaria was replaced with fresh water after every three days regularly.

Experimental site selection and water sampling

This project was planned to study the exposure effects of domestic waste and industrial effluents on water quality parameters and fish under laboratory conditions.

Faisalabad the so called "Manchester" of Pakistan has plastic, leather, textile, dying, printing, finishing, seizing industries, sugar mills and a large number of factories that release the variety of industrial discharges and municipal wastes into River Chenab through Chakbandi drain at Moza Thatta Muhammad Shah (Ahmad Wala) at latitude 31.570° and longitude 72.534°. Five sites of Chakbandi drain were selected for water sampling. These sites were namely, Akbar Abad Chowk Faisalabad, Bawa Chak Faisalabad, Chak- 188 J. B., Chak Sial 194 and Moza Thatha Muhammad Shah (End point of the drain entering into River Chenab) along the stretches of Chakbandi drain (Fig. 3.1). This drains, almost span more than 160 km of distance before joining the river Chenab. Water sampling were done from five selected sites along the length of drain by depth, centre, right and left banks. Representative water samples from each selected site were collected in pre-washed polypropylene bottles with polyethylene caps from the five selected sites.

Determination of 96h LC_{50} values

LC_{50} values for three fish species were determined separately according to their resistance and sensitivity to drain water concentrations. The period for LC_{50} determination was 96 hours (short term) and mortality data was recorded for this duration. During this duration fish behavior was also observed. The median sublethal concentration (LC_{50}) was calculated in acute toxicity trials following the probit analysis as described by Finny (1971). Proper aeration was supplied continuously to all the glass aquaria with electric air pump.

Experimental setup

In this study, after the determination of LC_{50} Chakbandi drain's composite water concentration of five selected sites was prepared. The dead fish was immediately removed from the test tank. The five sub-lethal dilutions (20%, 25%, 30%, 35%, 40%,) of this composite water concentration was applied to fingerlings approximately 11-13g of three fish species i.e. *Catla catla, Labeo rohita* and *Cirrhinus mrigala* under laboratory conditions in glass aquaria.

All the three fish species were distributed into two groups, one as sub-lethal exposure group (experimental) which was further subdivided into five sublethal concentrations (40-20%) and other as control group. 30 fish of the three fish species were distributed equally into 18 glass aquaria (80L). The experimental glass aquaria were aerated continuously with an air pump through well quipped capillary system. The percentage concentrations of drain water were prepared on the basis of volume to volume (*v/v)* ratio.

Methodology

Fish tissues i.e. liver and kidneys were taken by dissecting the control and experimental fishes for histopathological studies. Following procedure was adopted for histopathology (Sultana *et al*., 2016).

Following are the important steps for histopathological studies

I. Fixation: Organ tissues were kept in Bouin solution for 24 hrs. Solution consisted of aqueous picric acid, formalin and glacial acetic acid in a ratio (75: 20: 5) respectively.

2. Dehydration: After fixation, tissues slices were kept in tissue baskets with tagging. Dehydration was done in Alcoholic ascending grade series as 30% (for 30 minutes), 50 %(for 30 minutes), 70%, 90% (for 2 hours each) and 100% for 2 hours two times.

3. Clearing: After dehydration, tissue baskets were transferred to clearing agent, Xylene for 1 hour and 2 hours.

4. Infiltration: After clearing, tissue baskets were placed in molten paraffin wax for 45 minutes and 60 minutes at 58-69 °C in oven.

5. Embedding: Tissues were removed from baskets and embedded in molds with paraffin wax. Wax blocks with embedding tissues were freezed for solidification.

6. Sectioning: Tissues were cut at 2-3 micron by using Microtome. Ribbons were spread on glass slide containing adhesive material as glycerin and albumin. Sections were placed in oven at 37°C for overnight.

7. *Staining:* Wax was removed from sections with xylene for 2 minutes for 3 times. Hydration was done by immersing the tissues in descending series of alcohol for 1-2 minutes each. (100, 90, 70, 50 and 30%). Tissues were stained with hematoxylin stain for 2-5 minutes and then washed the slides under running tap water to remove excess stain. Counter stained with Eosin (1%) for 15 seconds to 2 minutes. Washed with water, dried in oven for 2 minutes. Mounted with Canada balsam.

8. Examination: Slides were examined under fluorescent microscope and photographed at 40x100 X objective lens. The histopathological changes were observed and compared with control.

Results

The present research work was designed to estimate the genotoxicity by using the histopathological biomarker approach in Indian major carps exposed to water containing domestic waste and industrial effluents that are being discharged through Chakbandi drain into River Chenab. The following results were observed.

Liver

Fish liver from control group showed the normal hepatocytes appearance, with central nuclei, arranged in cords around central region whereas cords of hepatocytes were separated by sinusoids. Liver of fish from control group also showed large number of glycogen granules (Fig. 1). Liver sections of different fish species after the exposure of different drain water concentrations for ninety days showed various histopathological changes. Fish exposed to DW-3 showed mild alterations such as ruptured central vein and few ruptured hepatocytes.

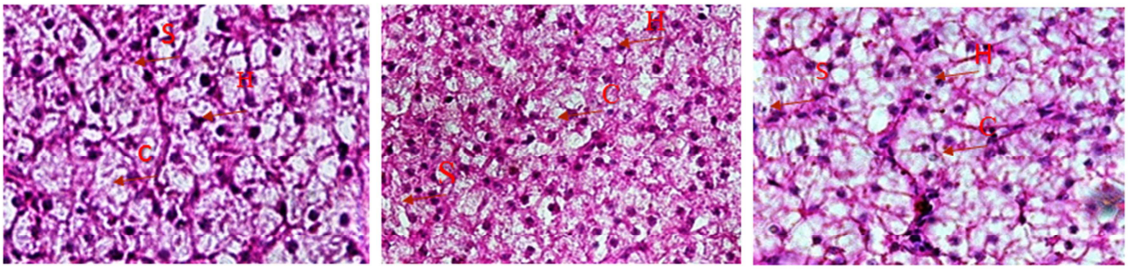
Hepatocytes arranged in cords were showing comparatively normal architecture. Liver of fish exposed to drain water concentrations DW-5 indicated dilated and congested central vein, ruptured hepatocytes with eccentric nuclei, degeneration of hepatocytes, vacuolization and necrosis.

Massive number of macrophage infiltration was noted in fish liver exposed to DW-5. Many cells degenerated and transformed to eosinophilic patches with no

nuclei or deeply stained nuclei whereas hepatocytes of treated groups also showed decreased glycogen granules/content (Fig. 2). Maximum abnormalities as severe necrosis, blood congestion and vacuolization were detected in the liver of *Cirrhinus mrigala* as compared to *Labeo rohita* and *Catla catla* exposed to drain water concentration DW-5, showing more sensitivity and susceptibility of *Cirrhinus mrigala* compared to other two fish species with respect to liver abnormalities (Fig. 2).

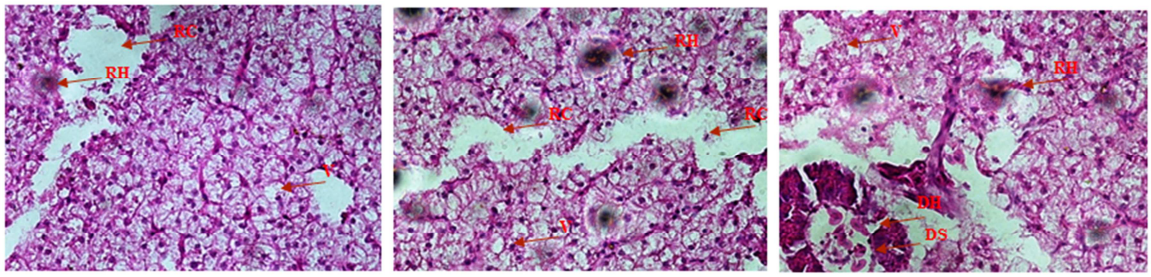
Kidneys

Fish kidney is a mixed organ. It performs the functions as hematopoetic, phagocytic and excretory organ and has distinct head and trunk region. Functional unit of kidney is nephron which is composed of glomerulus, proximal and distal renal tubules. Kidney from control group showed slightly spherical glomeruli with proper bowman space. Border of proximal tubules and lumen of distal tubules were prominent (Fig. 3). Kidney sections of treated groups with various drain water concentrations showed necrosis and degeneration of glomerulus with shrinkage of tubules and tubule lumen and decrease in hematopoietic tissue. Severe necrosis of tubules was noted in fish kidneys exposed to drain water concentration DW-5 (Fig. 4). Maximum abnormalities like severe necrosis, blood congestion and vacoulization were detected in the kidney of *Cirrhinus mrigala* as compared to *Labeo rohita* and *Catla catla* when exposed to drain water concentration DW-5. *Cirrhinus mrigala* showed more sensitivity and susceptibility as compared to other two fish species with respect to kidney abnormalities (Fig. 4).



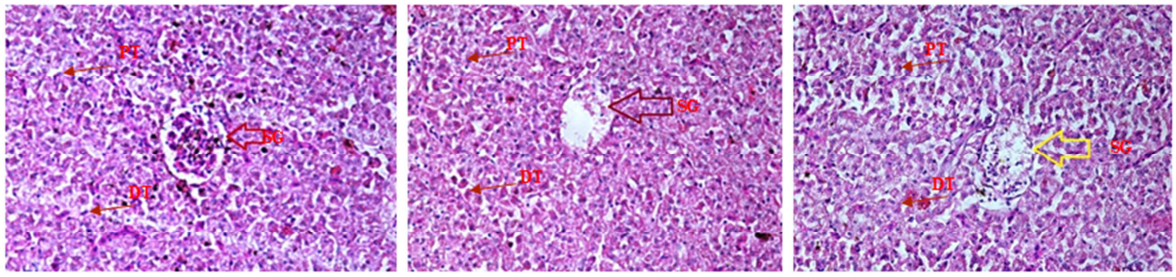
a. *Catla catla* b. *Labeo rohita* c. *Cirrhinus mrigala*

Fig. 1. Control water fish species showing normal liver tissues: (Hepatocyte cell (H): Sinosoids (S): Central nucleus) (C). Hematoxilin & Eosin stain, 400 x)



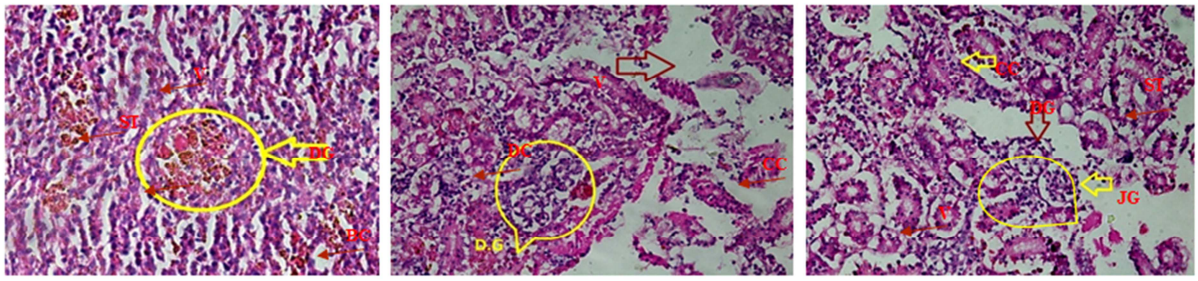
a. *Catla catla* b. *Labeo rohita* c. *Cirrhinus mrigala*

Fig. 2. Drain water treated fish species showing damaged liver tissues. (Damaged hepatocyte (DH): Ruptured central canal (RC): Vacuolization (V): Damaged sinosoids (DS). Hematoxilin & Eosin stain, 400 x)



a. *Catla catla* b. *Labeo rohita* c. *Cirrhinus mrigala*

Fig. 3. Control water fish species showing normal kidney tissues. (Spherical glomerulus (SG): Distal tubule (DT): Proximal tubule (PT) Hematoxilin & Eosin stain, 400 x).



a *Catla catla* b. *Labeo rohita* c. *Cirrhinus mrigala*

Fig. 4. Drain water treated water fish species showing damaged kidney tissue. (Degeneration of glomerulus (DG); Vacuolization (V): Jointed glomerulus (JG): Clubbing of cells (CC): Sharinkage of tubules (ST). Hematoxilin & Eosin stain, 400 x)

Table 1. Sub-lethal drain water concentration (%) applied to different fish species in glass aquaria.

Drain water concentrations	No. of *C. catla*	No. of *L. rohita*	No. of *C. mrigala*
Control water	10	10	10
DW- 1 (20%)	10	10	10
DW- 2 (25%)	10	10	10
DW- 3 (30%)	10	10	10
DW-4 (35%)	10	10	10
DW- 5 (40%)	10	10	10

Discussion

Histopathological biomarker approach is valuable for environmental risk assessment and genotoxicity evaluation in fish and of water body. The municipal waste and industrial effluents are continuously discharged through different drains into River Chenab, in southern Punjab, Pakistan. The contamination of fresh water with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threats to public water supplies but also the damages caused to the aquatic life (Sultana *et al.*, 2016; Jamdade & Gawande, 2017).

In the present study, the histopathological changes were observed from liver and kidney tissues of all the three drain water treated fish species. Maximum abnormalities as severe necrosis, blood congestion and vacuolization were detected in the liver of *Cirrhinus mrigala* as compared to *Labeo rohita* and *Catla catla* when exposed to concentration. *Cirrhinus mrigala* reflected more sensitivity and susceptibility than other two fish species with respect to liver abnormalities. The histological analysis in the control fish showed normal structure but in the treated fishes different abnormalities were observed.

Hepatic tissue of fish species exposed to drain water concentrations showed structural alteration. Prominent variations were observed in liver tissues of all the three drain water treated fish species as compared to control group. The fish liver tissues exhibited dilated sinusoids, damaged hepatocytes, vacuolar degeneration, degenerated hepatic cells and necrosis, dilated sinusoids, damaged hepatocytes, vacuolar degeneration and degenerated hepatic cells were noted.

These findings are in accordance with findings of Liebel *et al.* (2013) reported alterations in the histology of liver and gills with somatic indexes which were used for toxicity assessment. They analyzed aneurysms, hyperplasia, lamellar fusion and neoplasia in gills, hardening and macrophage infiltration in liver. They used histology of gills and liver as biomarker to detect the water quality parameters and health status of aquatic organisms. These findings are also in accordance with findings of Paul *et al.* (2014) who observed necrosis, degradation of hepatocytes, degeneration of blood vessels, distended sinusoids with pyknotic nuclei and vacuolation of cells. They found that the level of injuries in liver tissue was proportional to the concentrations of the metals used.

In the present study kidney sections of fish treated with various drain water concentrations showed necrosis and degeneration of glomerulus with complete obliteration of bowmen's space, shrinkage of tubules and tubule lumen and decrease in hematopoietic tissue. Severe necrosis of tubules was noted in fish kidneys exposed to drain water concentrations. Maximum abnormalities as severe necrosis, blood congestion and vacoulization were detected in the kidney of *Cirrhinus mrigala* as compared to *Labeo rohita* and *Catla catla* when exposed to DW-1 and DW-4 concentrations. *Cirrhinus mrigala* reflected more sensitivity and susceptibility than other two fish species with respect to kidney abnormalities.

The present study's findings are in accordance with findings of Latif *et al.* (2012) reported abnormalities in kidney reflected the degeneration of the glomerular tissue, necrosis and occlusion in tubular lumen.

These findings are in accordance with the findings of Faheem *et al.* (2016) investigated the histological effects in liver such as inflammation, edema, central vein congestion, degeneration and necrosis of hepatocytes. Kidney abnormalities identified were shrinkage and degeneration of tubules and obliteration of bowmen's space. Gills were detected to respond the stress by hyperplasia of mucous cells, clubbing and degeneration of secondary-lamellae. These findings are in accordance with the findings of several researchers (Faheem *et al.*, 2016; Tayyabah *et al.*, 2012).

Conclusion

Conclusively, histopathological changes in *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* is a useful tool to evaluate the impact of toxicity of xenobiotics in vital functions of a living organism. The prominent alterations were observed in liver, kidney, gills and muscle tissues of all the three fish species exposed to different drain water concentrations as compared to control group. Hence, the findings of this study are helpful for the evaluation of eco-toxicological impacts of pollutions reaching in aquatic fauna particularly fish and indirectly to human populations.

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