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Original article

Environmentally induced nephrotoxicity and histopathological alternations in *Wallago attu* and *Cirrhinus mrigla*

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ABSTRACT

Fish kidneys are sensitive to chemical changes in the freshwater ecosystem because they are directly and constantly exposed to chemicals dissolved in the water. This study evaluated nephrotoxicity in *Wallago attu* and *Cirrhinus mrigala* harvested from the Chenab River in an area of industrial and sewage waste disposal. Induced histological alternation data were correlated to the severity of environmental degradation in order to determine whether this biological system can be used as a tool for environmental monitoring programs. Kidneys from two fish species occupying different niches were collected and stored for 24 h in 10% formalin. Control fish were collected upstream of the polluted river area. Specimens were processed using topical histological methods. The major histological alterations observed in both species were renal tubule myxospora, hyperemia, glomerulonephritis, degeneration of renal tubule cells, dilation of glomerular capillaries, presence of pycnotic nuclei in the hematopoietic tissue, epithelial hypertrophy, vacuolization, reduced lumen of renal tubules, and shrinkage of glomeruli. Renal tubular atrophy, degeneration due to extensive degranulation, necrosis of glomeruli, glomerular expansion, absence of Bowman's space, hypertrophied nucleus, necrosis and hyalinization of the interstitium, clogging of tubules, and regeneration of tubules was also observed. *Wallago attu* exhibited the maximum incidence of moderate to severe changes and was defined as having the highest "histopathologic alteration index". These severe alterations were found to be related to environmental degradation, indicating the presence of stressors in freshwater. Control groups showed normal tissue morphology in the kidneys.

1. Introduction

A significant change in freshwater quality is attributable to the onset of the industrial revolution and the responsibility is slighted by a pessimistic anthropogenic attitude. This contamination results in anxiety, disorder, instability, and damage to ecosystem collectively referred to as pollution. Freshwater pollution has now become a major problem in the global context. It consists of two types: inorganic and organic. Pollutants which obstruct various biological functions of aquatic animals are inorganic in nature (Atli et al., 2015). These turbidity-causing impurities are precipitated salts, fine sand, soil, clay and industrial wastes such as dyes and metallic salts. Recent literature has revealed the increasing load of heavy metals in freshwater bodies. These metallic salts are directly associated with reproductive defects, nerve damage, increasing incidence of cancer and increased susceptibility to different diseases (Singla, 2015).

Biomarkers are biological response to any contaminants and indicates the change in the status of the organism (Chambers et al., 2002). The ecotoxicological approach to biomarkers used in fish to identify the changes in the lower levels of the biological organization before the populations are affected. Fish gills, kidneys and liver are considered as an important vital organ to use as a biomarker in determining the health of fish in the freshwater ecosystem (Bernet et al., 1999). The tissues and organs which are in contact with the foreign compounds have the potential to suffer the most and cause a damage when exposed to higher concentrations of chemical pollutants (Timbrell, 1991). Histological examination, as an indicator of exposure to pollutants, is a useful method for evaluating the degree of pollution (Bernet et al., 1999). In this context, the histological examinations carried out

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on the fish to determine the contamination in the aquatic ecosystems can provide a useful information about the health of these ecosystems.

In the case of aquatic animals such as fish, pollution leads to morphological and cytological changes in the liver (Ikram and Malik 2009; Deore and Wagh, 2012; Atli et al., 2015) kidney (Amin et al., 2013) and gills (Al-Mansoori, 2006). It causes nephron damage in the kidneys resulting in renal dysfunction (Nordberg et al., 2012). The kidneys are vital organs in the maintenance of pH and volume of body fluids as well as erythropoiesis (Iqbal et al., 2004). In fish, they maintain a water and electrolyte balance in order to provide a stable internal environment. Due to these properties, the kidneys are also an outstanding indicator of possible pollution and environmental stress in the vicinity of the fish (Hinton and Lauren, 1990). This project was planned to study the toxic effect of heavy metals and nephrotoxicity induced by pollutants and the responses of the carnivorous fish *Wallago attu* and the herbivorous fish *Cirrhinus mrigala,* which occupy different niches.

2. Materials and methods

2.1. Fish and water sampling

Adult fish specimens of each species (*Wallago attu* and *Cirrhinus mrigala*) were harvested from the River Chenab. Two sampling locations Meroki (Site 1) and Thali (Site 2) were selected as upstream sites to the "entrance of the Chakbandi Main Drain (CMD) into the Chenab River" in Tehsil and District Jhang for comparison with fish from Maral Wala (Site 3), Binoi Said Jaial (Site 4) and Dhanu Wala (Site 5) downstream of the entrance of the CMD. "Fishing was performed with the help of professional local fishermen by using gill nets and drag nets". Collected *Wallago attu* specimens ranged 750–1200 g and *Cirrhinus mrigala* ranged 700–1050 g in weight. Twenty-one fish specimens were collected for each fish species from each experimental site of the Chenab River. Water samples were also collected from every point of fish harvesting and analyzed for pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total dissolved solids (TDS), total suspended solids (TSS), salinity, conductivity, phenols, sulfates and other water-quality characteristics, according to the methods described by Boyd (1981) and selected heavy metals through an atomic absorption spectrophotometer.

2.2. Histopathology

Histopathology was performed by paraffin embedding methods because all required reagents are easily available, inexpensive and less toxic to humans. Fish were sacrificed to remove the kidneys and tissue sectioning and fixation (10% formalin) was performed quickly in order to strengthen and guard the tissue against degradation and cellular damage and to minimize autolysis (Ortiz-Ordoñez et al. 2011). Dehydration of tissue blocks was performed by a stepwise gradient series of isopropyl alcohol (IPA) solutions in a slow, step-wise method. Tissue blocks were infiltrated prior to sectioning with paraffin. "The paraffin equilibrated within the tissue block during infiltration and it occupied all of the space in the tissue that was initially filled by IPA, and subsequently the tissue solidified and was embedded within a small cube of paraffin". Paraffin was removed before the section staining by soaking in a cleaning agent three times for 1–2 min and washed with tap water for 30 s. After clearing the paraffin, only tissue remained adhered to the slide. Dehydration was performed by 70%, 85%, 100% alcohol for 30 s each gradually. Tissue sectioning was performed using a microtome. The thin sections were cut from the surface of the paraffin cubes with a very accurate thickness of 8 μm. The sections became slightly crinkled during sectioning and in order to overcome these wrinkling conditions samples were floated on water and then transferred to the labeled slides. Histological sections were stained with hematoxylin to obtain photomicrographs and analyze the results (Bernet, et al., 1999; Ortiz-Ordoñez et al., 2011). Photomicrographs were taken from stained sections under 40×10× magnification using a microscope (Nikon DS- fi2 MODEL ECLIPSE Ci-L).

2.3. Statistical analysis

For data regarding pollution studies, the mean, standard error, and analysis of variance (ANOVA) were calculated using SPSS 9 for PC. The mean values of water quality parameters were compared through DMR tests ($p < 0.05$).

3. Results

Wallago attu and *Cirrhinus mrigala*, harvested from the contaminated sites of the River Chenab were analyzed for genonephrotoxicity. The heavy metals and other Physio-chemical in this study were observed higher than the WHO permissible limits defined for freshwater reservoirs (Table 1).

The toxin-free group of fish designated as control displayed normal arrangements of cells. No kidney histopathological changes were observed in this group. Typical brush borders and basal nuclei were present in epithelial cells while distal segments had no brush borders and central nuclei. Normal glomeruli structures and thin intracapsular space in Bowman's capsule were observed. Normal symmetry of chromaffin cells, epithelial cells and tubules and normal structures of glomeruli and Bowman's capsule were observed, but in some fish, slight clogging of the tubules was recorded. The fish procured from contaminated sites (downstream to CMD) showed significant histopathological alterations. These pollutants severely affected the kidneys of fish.

Photomicrographs of the histopathology of the kidneys of *W. attu* from control sites showed a normal kidney architecture (Fig. 1). Histopathology of *C. mrigala* kidneys collected from non-polluted sites showed normal nephron structures with slight congestion of tubules (Fig. 2). Normal "renal tubule, glomerulus, Bowman's capsule, hematopoietic tissue and epithelial cell structures were observed in both fish species collected from control sites".

Histopathology of *W. attu* kidneys collected from polluted areas of the Chenab River showed epithelial hypertrophy, "vacuolization, reduced lumen of renal tubules and shrinkage of glomerulus. Renal tubular atrophy, sites of degeneration due to extensive degranulation and necrosis of glomeruli" were also observed (Fig. 3). Glomerulus expansion, absence of Bowman's space, hypertrophied nuclei, and regeneration and occlusion of tubule lumen were also noted in photomicrographs (Fig. 4). Necrosis of the hematopoietic interstitial tissue and vacuolar degeneration of renal tubules were observed (Fig. 5).

Photomicrographs of the kidney histopathology of *C. mrigala* harvested from the contaminated site of River Chenab (downstream MD) also exhibited abnormalities such as renal tubule myxospora, hyperemia, and glomerulonephritis (Fig. 6). Degeneration of renal tubule cells, "dilation of glomerular capillaries and presence of pycnotic nuclei in the hematopoietic tissue were seen in photomicrographs of tissues" (Fig. 7). Photomicrography revealed degeneration of tubules including vacuolization, necrosis and severe clogging of tubules (Fig. 8). Kidneys of *C. mrigala* were found to be more susceptible to pollution, indicating more abnormalities in comparison to *W. attu.* More singularities were also observed such as glomerular necrosis and dilation of glomerular capillaries.

Table 1
Comparison of means of water quality parameters of different sites* along the Chenab River (mean ± SE).

WQPs	Site 1	Site 2	Site 3	Site 4	Site 5
pH	7.50 ± 0.05 a	7.30 ± 0.08 a	9.69 ± 0.05b	9.14 ± 0.04 b	8.90 ± 0.06 c
BOD (mg/L)	32.71 ± 1.06 a	37.29 ± 0.61 b	93.00 ± 0.93 c	84.14 ± 1.06 d	78.43 ± 1.36 e
COD (mg/L)	42.43 ± 0.65 a	38.00 ± 0.72 b	141.86 ± 1.84 c	135.57 ± 2.03 d	129.29 ± 1.96 e
Hardness (mg/L)	180.00 ± 5.35 b	205.71 ± 3.69 b	508.57 ± 4.04 d	548.57 ± 8.57 c	581.43 ± 11.84 c
Conductivity (μS/cm)	642.86 ± 17.0 b	828.57 ± 18.4 a	1157.14 ± 29.7 c	1742.86 ± 25.4 d	2364.29 ± 26.1 e
TSS (mg/L)	1.159 ± 0.034 b	1.046 ± 0.022 b	2.319 ± 0.055 d	1.850 ± 0.044 e	1.476 ± 0.041 c
TDS (mg/L)	1.046 ± 0.026 a	1.084 ± 0.029 a	1.841 ± 0.054b	2.007 ± 0.072c	2.054 ± 0.089c
TS (mg/L)	2.204 ± 0.059 a	2.130 ± 0.049 a	4.153 ± 0.106b	3.857 ± 0.11 d	3.530 ± 0.129c
Ni (mg/L)	0.034 ± 0.003 a	0.066 ± 0.006 a	0.242 ± 0.014 b	0.213 ± 0.003 b	0.143 ± 0.002 d
Cr (mg/L)	0.038 ± 0.003 a	0.069 ± 0.006 a	0.283 ± 0.006b	0.273 ± 0.003b	0.206 ± 0.003c
Mn (mg/L)	0.040 ± 0.002 a	0.136 ± 0.019b	0.264 ± 0.004 c	0.189 ± 0.003 d	0.158 ± 0.002 e
Co (mg/L)	0.615 ± 0.021 a	0.721 ± 0.010 b	1.091 ± 0.005 c	1.029 ± 0.002 c	0.799 ± 0.002 e
Pb (mg/L)	0.074 ± 0.004 b	0.046 ± 0.004 b	0.867 ± 0.018 d	0.959 ± 0.009 d	1.647 ± 0.006 c
Hg (mg/L)	<0.001 ± 0.00 a	<0.001 ± 0.00 a	0.025 ± 0.001c	1.04 ± 0.006b	0.701 ± 0.003 d
Zn (mg/L)	0.039 ± 0.004 a	0.034 ± 0.004 a	1.05 ± 0.008 b	0.87 ± 0.020 c	0.912 ± 0.014 c
Sn (mg/L)	0.004 ± 0.001 a	0.003 ± 0.001 a	0.320 ± 0.003 b	0.244 ± 0.003 c	0.334 ± 0.010 b
Cu (mg/L)	0.049 ± 0.001 a	0.029 ± 0.002 a	0.907 ± 0.003 c	0.863 ± 0.003 b	0.826 ± 0.010 b

Means sharing a similar letter in a row belonging to particular parameter are statistically non-significant (P > 0.05). COD; Chemical Oxygen demand, BOD; Biochemical Oxygen demand, TSS; Total suspended solids, TDS; Total dissolved solids, TS; Total solids. *31°34′14.0″N 72°32′02.8″E.

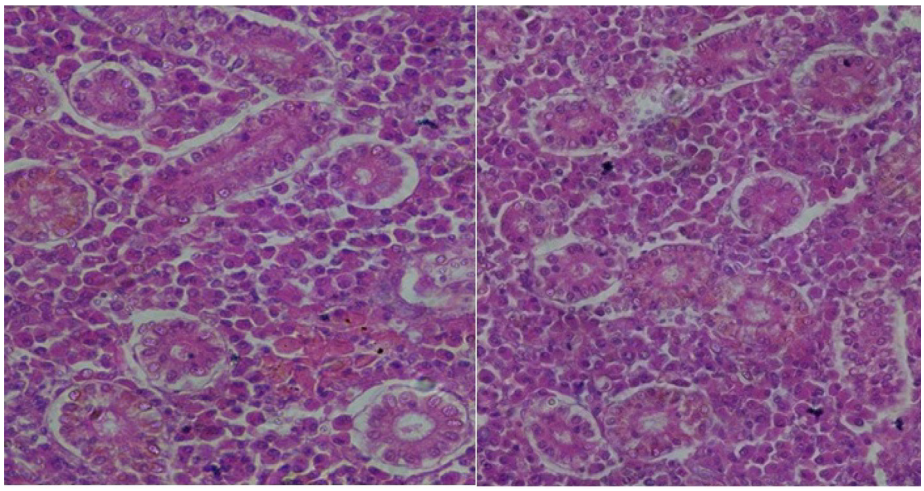

Fig. 1. Photomicrograph for histopathology of fish *Wallago attu* collected upstream of the entrance of Chakbandi Main Drain (control) into the Chenab River indicating normal kidney structures (40×10×).

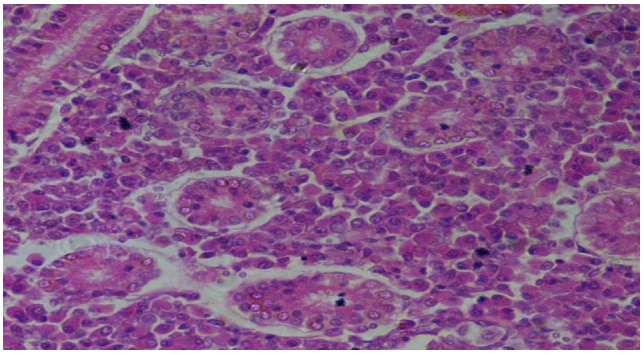

Fig. 2. Photomicrograph for histopathology of *Cirrhinus mrigala* collected upstream of the entrance of Chakbandi Main Drain (control) into River Chenab indicating normal kidney structures (40×10×2× magnification).

Necrosis and hyalinization of the interstitium, clogging of tubules and necrosis (Fig. 9) was found to be more prominent. Hydropic swelling of tubules (Fig. 10) and tubular necrosis were major abnormalities due to pollution seen through photomicrography of kidney tissues.

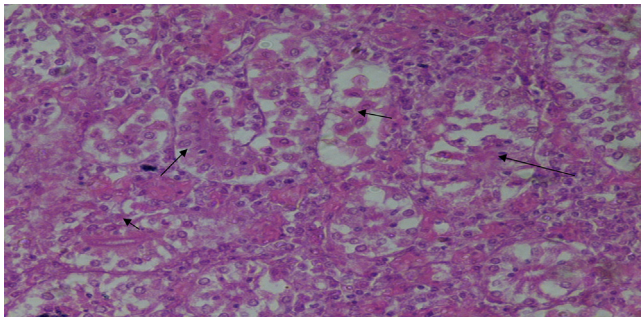

Fig. 3. Photomicrograph of *Wallago attu* kidney collected from polluted site of the Chenab River indicating glomerulus expansion, absence of Bowman's space and hypertrophied nucleus (40×10×).

4. Discussion

Only monitoring chemical accumulation in an ecosystem is not enough to determine the effects of these contaminants on the organism, population and communities (Velmurugan, 2011). In terms of sub-lethal levels of a chemical, the response of the organ-

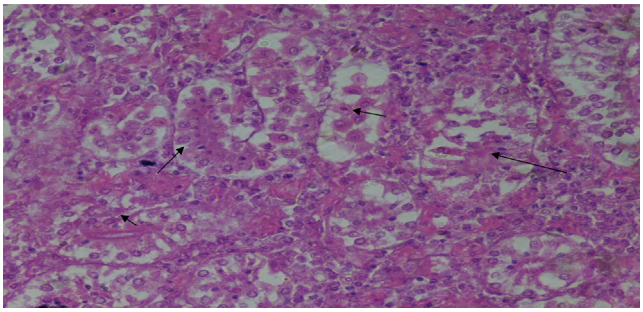

Fig. 4. Photomicrograph for histopathology of *Wallago attu* fish collected from polluted site of Chenab River indicating glomerulus expansion, absence of Bowman's space and hypertrophied nucleus (40×10× magnification).

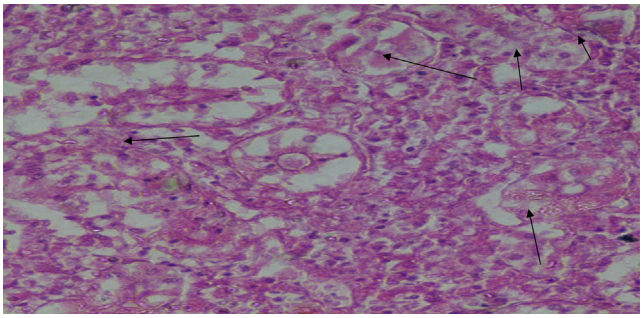

Fig. 5. Photomicrograph of *Wallago attu* kidney collected from a polluted site of the Chenab River indicating renal tubular atrophy, necrosis of glomerulus and site of degeneration due to extensive degranulation (60×10× magnification).

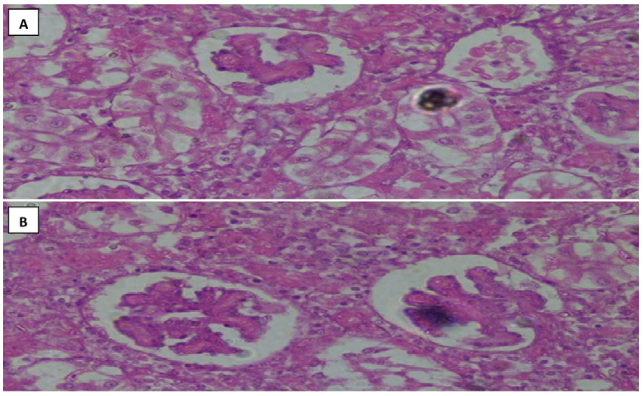

Fig. 6. Photomicrograph of kidney of *Cirrhinus mrigala* collected from polluted site of the Chenab River indicating renal tubules myxospora and hyperemia (A) Glomerulonephritis (B) (40×10×3× magnification).

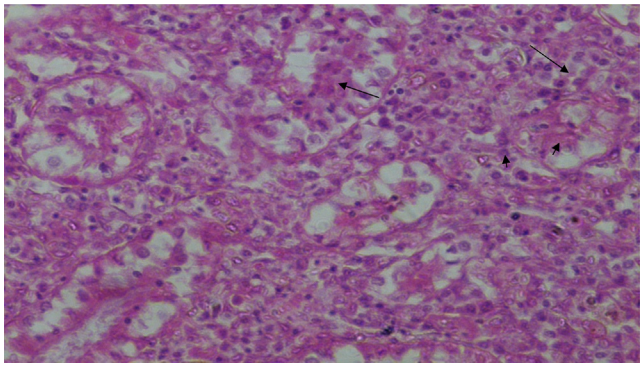

Fig. 7. Photomicrograph of histopathology of fish *Cirrhinus mrigala* collected from polluted site of the Chenab River indicating degeneration in the epithelial cells of renal tubule and pycnotic nuclei in the hematopoietic tissue (40×10×2× magnification).

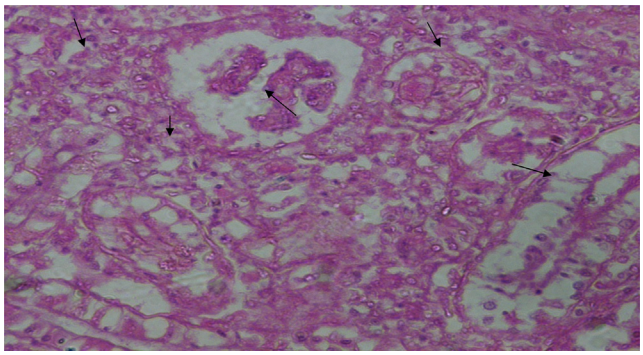

Fig. 8. Photomicrograph of histopathology of fish *Cirrhinus mrigala* collected from a polluted site of the Chenab River indicating degeneration of tubules including vacuolization (40×10×2×).

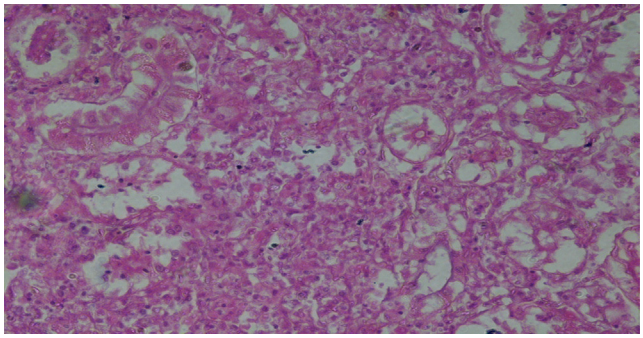

Fig. 9. Photomicrograph of histopathology of fish *Cirrhinus mrigala* collected from a polluted site of the Chenab River indicating Necrosis, hyalinization of interstitium and clogging of tubules (40×10× magnification).

ism to contamination; as used in medical diagnostics in humans or as a veterinarian in clinical toxicology; can only be determined through biological, physiological and biochemical parameters (Lagadic et al., 2000). Biomarkers, are used to identify negative biological responses to environmental anthropogenic toxins in the organism (Bucheli and Fent, 1995).

The greatest structural damages resulting from exposure to pollutants may be in target organs. As a result of the exposure to pollutants, the histological structure may change and physiological stress may occur. This stress can cause some changes in metabolic functions. The changes in functions are initiated by changes in cellular level and tissues.

The teleost kidney is one of the first organs to be affected by aquatic pollutants. In environmental screening, the use of histopathological biomarkers to inspect specific vital organs such as kidney, gills, muscles and liver has important benefits. Fish species respond to the unmediated effects of toxic pollutants along with secondary effects caused by environmental stress (Rashed, 2001; Elia et al., 2002; Thophon et al., 2003; Velmurugan et al., 2007; Abdel-Khalek, 2015). Reddy and Rawat (2013) and Muñoz et al. (2015) verified the assessment of field pollution through histopathological alterations affected by different pollutants which corroborate the findings of this project. They also indicate in their study that histopathology has the propensity to "gauge the initial effects and responses to acute exposure of toxins and stressors". Similar findings were also reported by Pontes-Viana et al., 2013 and Ortiz et al. (2003).

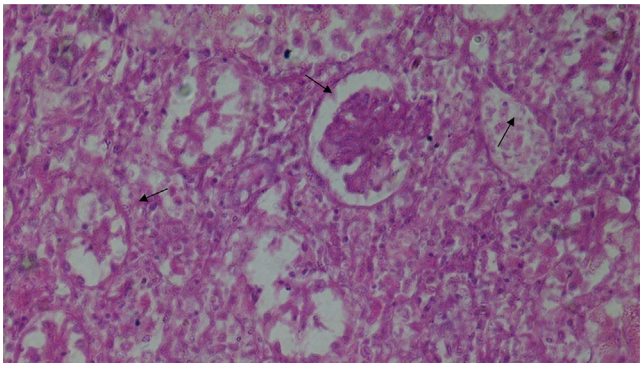

Fig. 10. Photomicrograph of kidney histopathology of fish *Cirrhinus mrigala* collected from a polluted site of the Chenab River indicating glomerular necrosis and hydropic swelling of tubules (40×10× magnification).

In this experiment, photomicrographs for the histopathology of kidney structures in case of control site fish showed normal interrenal, chromaffin, and hemopoietic tissues, renal corpuscle, renal tubule, glomerulus, "Bowman's capsule, proximal tubule and distal tubule" structures while in the case of polluted site fish many abnormalities such as Necrosis of the hematopoietic interstitial tissue, vacuolar degeneration of renal tubules, narrowing of the tubular lumen, glomerulonephritis and renal tubular atrophy were found and these results were found to be in line with the findings of Subhadra and Shelley (1994), Cengiz, (2006) and Sultana et al. (2016). Findings of this project in response to freshwater pollution, such as glomeruli expansion, hypertrophied nucleus, occlusion of the lumen of tubules, absence of Bowman's space, degeneration of renal tubular cells, regeneration of tubules, "dilation of capillaries in the glomerulus", severe clogging of tubules, glomerular shrinkage, and "necrosis of the hematopoietic interstitial tissue" substantiate the findings of Benli et al. (2008), Drobac et al. (2016), Cuevas et al. (2016), Tabassum et al. (2016), and Pereira et al. (2017). Histopathological alternations in the kidney structures of the fish species in this study also validate the findings of Ortiz et al. (2003), Thophon et al. (2003), Van et al. (2004), Olojo et al. (2005), Velmurugan et al. (2007), Peebua et al. (2008), and Mishra and Mohanty (2008) who reported nephrotoxicity in response to heavy metals.

Drishya et al. (2016) and Mohamed (2009) reported that a better picture of the fish's health is provided by histology. They also validate it as an integrated parameter for providing an effective monitoring of the effects of water pollution.

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