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A study on risk assessment of effect of hematoxylin dye on cytotoxicity and nephrotoxicity in freshwater fish: Food and water security prospective research

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ABSTRACT

The cytotoxicity in freshwater fishes due to different industrial dyes in industrial effluents is a major worldwide issue. Hematoxylin dye has a wide range of uses in textile industries and laboratories. This study was aimed to evaluate the toxic effects of hematoxylin's sublethal effect in vitro in *Cirrhinus mrigala*. The fish was exposed to different grading concentrations of dye in the aquarium. Fish were sacrificed and dissected to remove the kidney after exposure to hematoxylin dye for specific time intervals. Nephrotoxicity and cytotoxicity induced by this dye were detected through histopathology by using the paraffin wax method. Immediate mortality of fish was noticed against the exposure to 0.08 g/L (LC₅₀) concentration of dye, but at 0.008 mg/L and 0.018 mg/L, it showed tremendous tissue damage in the kidneys, significant reduction in fish growth. This dye induced many alterations in the kidney such as tubular degeneration, vacuolation, shrinkage of a glomerulus, reduced lumen, congestion in the kidney, glomerulonephritis, absence of Bowman's space, necrosis of the hematopoietic interstitial tissues, clogging of tubules, necrosis in the glomerulus and increased space between glomerulus and Bowman's capsule. Although this dye has a wide range of biological and industrial applications, a minute amount of hematoxylin released in effluents is quite toxic to aquatic fauna.

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1. Introduction

Dyes are toxic substances having carcinogenic, cytotoxic, genotoxic, and neurotoxic effects on aquatic organisms (Sabnis et al., 2010). They are present in high concentrations in textile wastewater. Untreated discharge from textile industries into water bodies causes acute freshwater pollution by deteriorating the water quality, making it unfit for aquatic life (Verma, 2011). Freshwater pollution caused by textile dyes is a worldwide issue. These dyes are in close contact with fishes through gill respiration. Fish is a sus-

ceptible organism to a wide range of toxicants (Nemcsók et al., 1987). They are valuable models for evaluating various toxicants' effects in their environments (Gavilán et al., 2001). Biological modifications are referred to as biomarkers in fish associated with the introduction or impacts of contaminants. The use of these biomarkers has led to outstanding environment risk assessment outcomes (Oost et al., 2003; Dai et al., 2020). Histological studies are essential to finding the degree of pollution and are considered valuable tools that can be used to marker chronic and sub-lethal effects of freshwater pollution (Bernet et al., 1999; Hussain et al., 2019).

Contaminants enter into fishes' organs and damage the biochemical and hematological process (Nemcsók et al. 1987). Specific target organs like kidneys, liver, and gills can be used for environmental screenings. These organs perform vital functions like excretion, respiration, distribution, and biotransformation of xenobiotics in the fish (Gernhöfer et al., 2001). These toxicants can cause nephron damage in the kidneys resulting in renal dysfunction (Nordberg et al., 2012). Such organs provide a stable internal envi-

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ronment by maintaining body fluids and keeping water and electrolyte balance (Iqbal et al., 2004; Dai et al., 2020). Due to these reasons, the kidneys serve as an excellent indicator of environmental stress to fishes (Hinton and Lauren, 1990), while histopathological analysis indicate a tremendous biomarker due to its broad estimation (Perera and Pathiratne, 2010). This study was planned to assess in-vitro cytotoxicity and nephrotoxicity induced in *Cirrhinus mrigala* due to hematoxylin dye.

2. Material and methods

2.1. Collection of fish and acclimatization

Fish *Cirrhinus mrigala* of small size in the range of 20–30 g were procured from a private pond located at Bhalair Road, Sangla Hill, Pakistan. Fish specimens were acclimatized for four days in laboratory conditions with continuous aeration in glass aquaria before exposure to hematoxylin. Fish were fed with artificial feed according to 5% of their body weight. Aquarium water was changed daily to remove excreta. Fish weights were recorded before and after application of dye for every 15 days of the exposure period.

2.2. Experimental design

Fish specimens were classified into five groups for exposure to dye after taking their morphometric measurements. Twenty healthy specimens were selected for each trial of exposure. Hematoxylin was procured from Sigma-Aldrich, UK. The dye was dissolved in distilled water and diluted to prepare five different experimental concentrations viz., 0.001 mg/L, 0.002 mg/L, 0.004 mg/L, 0.008 mg/L, 0.018 mg/L. The five different fish groups were exposed to these experimental concentrations in triplicate. The control group of twenty fish specimens were kept in a dye-free aquarium for control. Water quality parameters such as dissolved oxygen, pH, and temperature were maintained properly during the whole period of the experiment (Bernet et al., 1999; Ameer et al., 2012). LC₅₀ was calculated by exposing fish to different hematoxylin dye concentrations for 24, 48, 72, and 96 h.

2.3. Histopathology

Fish were dissected to remove kidneys, and samples were prepared for histopathological evaluations by paraffin embedding method, and tissues were sectioned and fixed immediately to avoid autolysis (Ortiz et al., 2003). Dehydration of tissues was conducted in a slow step-wise method using 70% IPA, 85% IPA, 95% IPA, and 100% IPA concentrations of Isopropyl alcohol (IPA) within 1 h (Chavan and Muley, 2014). Tissue blocks were infiltrated with paraffin before sectioning. For infiltration, paraffin was equilibrated with a block of tissue. Tissue was solidified and embedded within a small paraffin cube (Bernet et al., 1999; Ameer et al., 2012). Tissue sections were prepared by using a microtome. Before the section staining, paraffin cleaning agent xylene was used three times for 1–2 min. The tissues were further processed and analyzed (Ameer et al., 2012; Chavan and Muley, 2014). After the staining of histological sections by hematoxylin (Bernet et al., 1999), photomicrography of stained sections was performed under a microscope ("Nikon DS-fi2 ECLIPSE Ci-L") at 40x and 60x magnification.

2.4. Statistical analysis

The data collected on growth performance was statistically analyzed and one-way analysis of variance was performed by using SPSS 9 software. One-way analysis of variance was performed.

The means were compared with DMR test ($p < 0.05$). Graphs were prepared using Microsoft Excel (2007).

3. Results

Fish *Cirrhinus mrigala* exposed to different concentrations of (0.001, 0.002, 0.004, 0.008, and 0.018 mg/L) of hematoxylin dye and it showed a significant reduction in the weight *C. mrigala*. LC₅₀ was worked out as 0.08 g/L, which showed immediate mortality of almost 50% of the fish after exposure to this dye. Although all the fish groups were fed 5% of their body weight with similar commercial feed. The highest weight reduction (average means) was reported in this trial group exposed to 0.018 mg/L of dye, and it caused a significant reduction in fish weight even at lower concentrations of hematoxylin. The weight of fish frequently decreased with an increase in the concentrations of dye (Fig. 1).

Typical structures and arrangements of cells were observed in the dye-free control groups. Photomicrograph of kidney tissues indicated that no histopathological alterations were observed. Normal structures of bowmen's capsule, glomeruli, thin inter capsular space, and average standard arrangement patterns of epithelial cells, chromaffin cells, and tubules were observed (Fig. 2). While the groups of fish treated with different dye concentrations displayed various histopathological alterations in fish's kidneys. The experimental group (TG1) treated with 0.001 mg/L concentration of dye showed slight degeneration of glomerulus and mild necrosis in the tubule (Fig. 3). Photomicrograph of the experimental group treated with 0.002 mg/L concentration (TG2) of dye showing degeneration of tissue and shrinkage of the glomerulus (Fig. 4) while experimental group (TG3) treated with 0.004 mg/L concentration of dye degeneration in the epithelial cells of the renal tubule, degeneration of tubules, necrosis in kidney and absence of bowmen's space (Figs. 5 and 6). The experimental group treated with 0.008 mg/L concentration (TG4) of dye reduced lumen of tubules, shrinkage of the glomerulus, and Glomerulonephritis (Figs. 7 and 8). Photomicrograph of the experimental group (TG4) treated with 0.018 mg/L concentration of dye showed increased per tubular space and clogging of tubules and degeneration of tubules, necrosis in kidney and absence of bowmen's space (Figs. 9 and 10). Significant mortality of fish was seen at 0.08 g/L (LC₅₀) of dye, but at its lower concentrations (0.001, 0.002, 0.004, 0.008, and 0.018 mg/L), it showed tremendous tissue damage in the kidneys and significant reduction in fish growth.

4. Discussion:

Urbanization and industry generate effluents and discharge them into freshwater bodies (Van-Vuren et al., 1999). These wastes contact freshwater organisms, especially fish, and induce noxious effects (Roopadevi and Somashekar, 2012). Such harmful effects are cytotoxic, nephrotoxic (Gbem et al., 2001), and genotoxic (Villela et al., 2006). Textile effluents contain various toxic dyes to deteriorate water quality and make it unfit for aquatic organisms, especially for fish (Verma, 2011). Histology provides a more comprehensive assessment of the health of the organisms and a useful tool to monitor the effects of introduction to environmental pollutants. The histopathological biomarkers found very useful against toxicants' exposure in vital organs like kidneys, muscles, liver, and gills. The teleost kidney is one of the primary organs to be affected by aquatic pollution (Oliveira et al., 2013). Fish species counter to the unmediated special effects of noxious pollutants next to the secondary effects of tension (Mohamed, 2009; Perera and Pathiratne 2010; Reddy and Rawat 2013). Histopathological alterations in fish are due to the presence of stressors and pollutants in the contaminated water (Chen et al. 2001; Abdel-

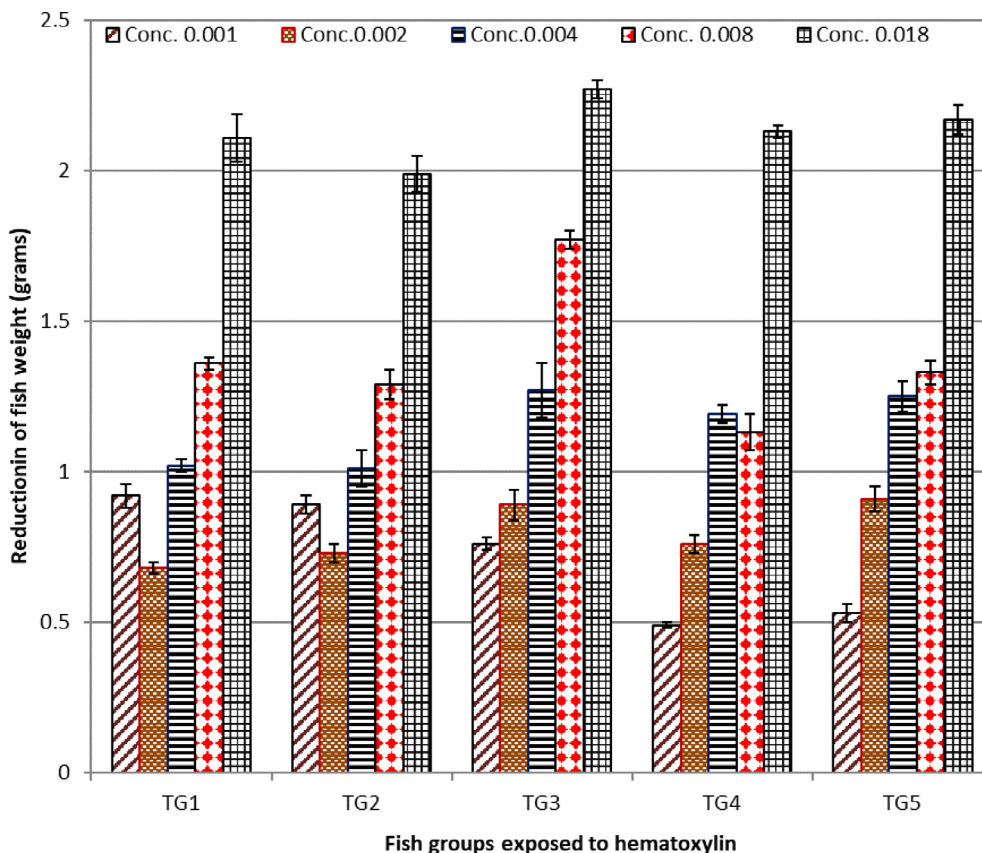


Fig. 1. Weight (grams) reduction in all experimental groups of fish in response to all (0.001, 0.002, 0.004, 0.008 and 0.018 mg/L) concentrations of hematoxylin dye.

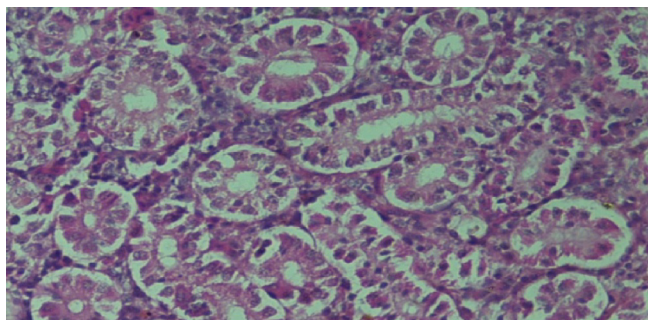


Fig. 2. Histopathology of fish *Cirrhinus mrigala* shows the kidney's standard structure in the control group (40 × 10x).

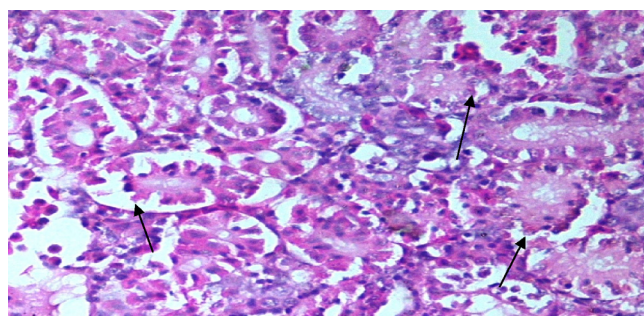


Fig. 4. Histopathology of fish *Cirrhinus mrigala* showing degeneration of tubules and shrinkage of glomerulus when exposed to 0.002 mg/L concentration of hematoxylin dye (40 × 10x).

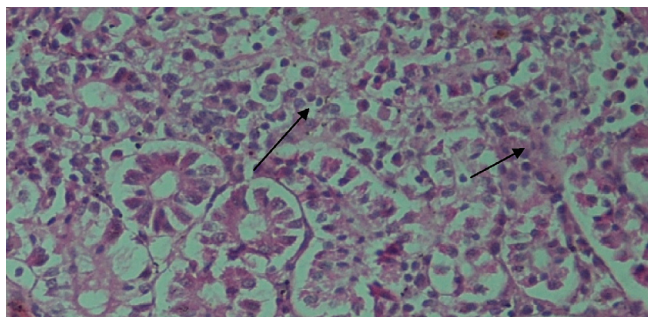


Fig. 3. Histopathology of fish *Cirrhinus mrigala* showing degeneration of glomerulus and mild necrosis in the tubule when exposed to 0.001 mg/L concentration of hematoxylin dye (40 × 10x).

Moneim, 2012; Paulo, 2012; Pirbeigi et al. 2016; Hussain et al. 2020).

The findings of the current study that textile dyes induce toxicity in freshwater fish corroborate Roopadevi and Somashekar (2012) results and Sreedevi and Chitra (2014). Olaganathan et al. (2013), De-Oliveira et al. (2016), and Aksu et al. (2017) also demonstrated that prolonged exposure to textile dyes induces changes in fish. Oliveira et al. (2013), Amte and Mhaskar (2013), De-Oliveira et al. (2016), and Rocha et al. (2017) in their study confirmed that textile dyes stimulate toxicity in fish. Prashanth (2011) used histopathological techniques to determine tissue alterations in the kidney of fish *Cirrhinus mrigala* in response to environmental toxicants. Paulo (2012) and Vijay et al. (2015) also used histological techniques to determine the toxic effects of textile dyes. Barot

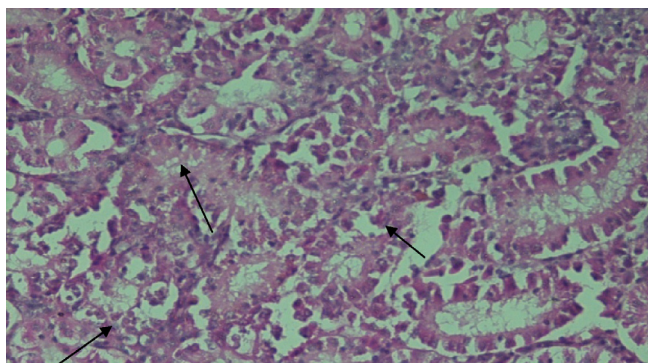


Fig. 5. Histopathology of fish *Cirrhinus mrigala* showing degeneration in the epithelial cells of renal tubule and necrosis in the kidney when exposed to 0.004 mg/L concentration of hematoxylin dye (40 × 10x).

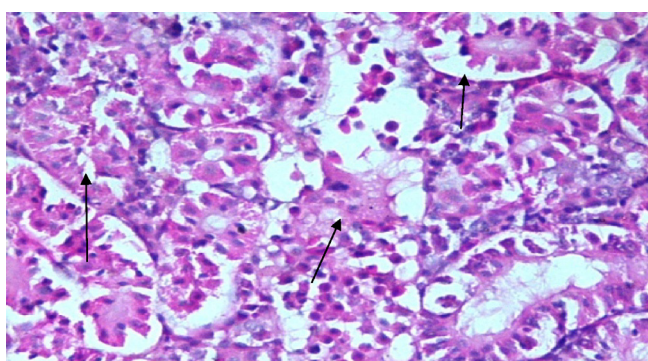


Fig. 6. Histopathology of fish *Cirrhinus mrigala* showing degeneration of tubules and absence of bowmen's space when exposed to 0.004 mg/L concentration of hematoxylin dye (40 × 10x).

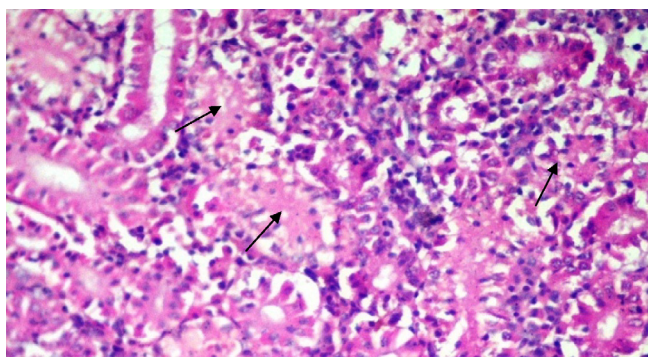


Fig. 7. Histopathology of fish *Cirrhinu mrigala* showing reduced lumen when exposed to 0.008 mg/L concentration of hematoxylin dye (40 × 10x).

(2015) revealed the induction of toxicity and tissue alteration in fishes.

Barot (2015) reported similar results in *Catla catla* similar to the current research project that hematoxylin dye induced alterations in the kidney of *Cirrhinus mrigala* like shrinkage of glomerulus and degeneration of renal tubules. These alterations and tissue damage increases with increasing concentrations of hematoxylin dye. Kaur and Kaur, (2015) reports similar results. Contraction of the glomerulus decreased lumen, and degeneration of nephrons was due to interference of dyes in the filtration process in the kidneys of fish (Barot and Bahadur. 2013; Dai et al., 2020). Findings of the current study in response to textile dye such as shrinkage of the glomerulus, necrosis, glomerular degeneration, and reduced space

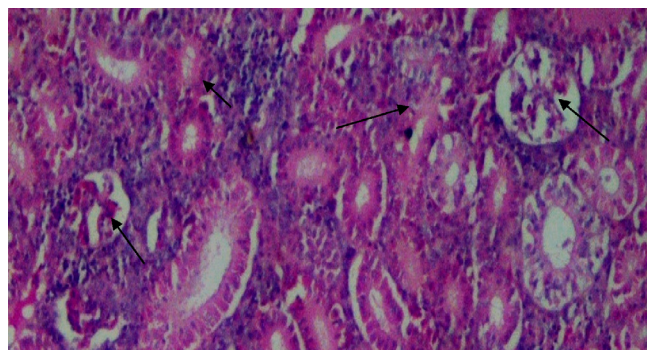


Fig. 8. Histopathology of fish *Cirrhinus mrigala* showing shrinkage of glomerulus Glomerulonephritis when exposed to 0.008 mg/L concentration of hematoxylin dye (40 × 10x).

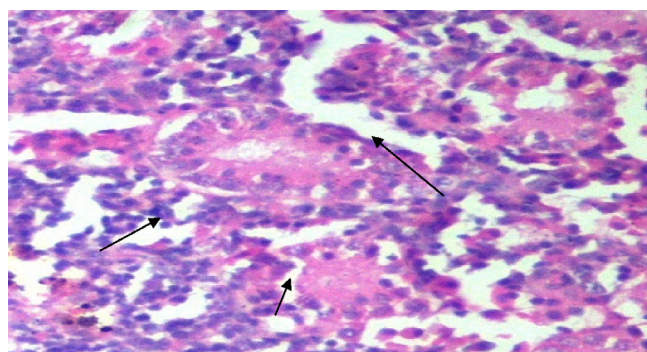


Fig. 9. Photomicrograph for histopathology of fish *Cirrhinus mrigala* showing increased per tubular space when exposed to 0.018 mg/L concentration of hematoxylin dye(40 × 10x).

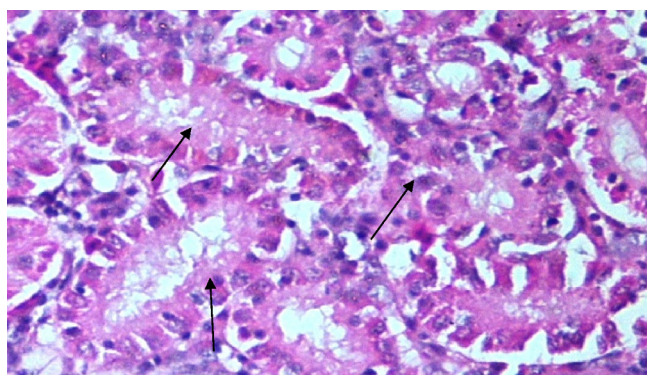


Fig. 10. Histopathology of fish *Cirrhinus mrigala* showing necrosis in the glomerulus and clogging of tubules when exposed to 0.018 mg/L concentration of hematoxylin dye (40 × 10x).

of Bowman's space in kidney were due to environmental contaminants (Ayas et al. 2007; Ashry and Mahmoud 2013; Massar et al. 2014; Brraich and Kaur 2017; Hussain et al., 2019). In another study by Saenphet et al. (2009), histopathological alterations such as contraction of the glomerulus and reduced lumen in the kidney of *Cirrhinus mrigala* were reported in exposure to different concentrations of dyes.

5. Conclusion:

It has been concluded that hematoxylin caused tubular degeneration, vacuolation, shrinkage of a glomerulus, reduced lumen,

congestion in the kidney, glomerulonephritis, absence of Bowman space, necrosis of the hematopoietic interstitial tissues, clogging of tubules, necrosis in the glomerulus and increased space between glomerulus and bowmen's capsule. Although this dye has a wide range of biological and industrial applications, a minute amount of hematxylin released in effluents is quite toxic to aquatic fauna.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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