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HISTOPATHOLOGICAL CHANGES IN TESTIS OF *RASBORA DANICONIUS* EXPOSED TO PAPER MILL EFFLUENT

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ABSTRACT: This article studies the histopathological effects of paper mill effluent on the testes of the freshwater fish, *Rasbora daniconius*. In this experiment, freshwater fish, *Rasbora daniconius*, were exposed to lethal concentrations of 9.5% (96-hour LC_{50}) for 96 hours and sublethal concentrations [1.9% (1/ 5) and 0.95% (1/10) 96-hour LC_{50}] for 30 days. A second group of fish without exposure to paper mill effluent was treated as a control. Control testes appeared structurally normal. Fish testes exposed to a 96-hour lethal concentration of 9.5% (LC_{50}) paper mill effluent showed apparent degeneration of lobules, necrosis and vacuolation of the testicular lumen and connective tissues. At a concentration of 0.95% paper mill effluent for 30 days of exposure, significant changes were observed in the seminiferous tubules: necrosis and vacuolated testicular lumen, disruption of tubule boundaries, cellular hypertrophy and absorption of spermatozoa. After exposure to a concentration of 1.9% of paper mill effluent for 30 days, distinct lesions appear such as rupture of tubule boundaries and liquefaction at the spermatogonium stage with blood hemorrhage in several locations. Complete sperm absorption with cellular vacuolation was also observed. The interstitial cells were found to disintegrate.

Key words: *Rasbora daniconius,* Paper effluent mill, Testes, and histopathology**.**

INTRODUCTION

Research on reproductive biology is important for assessing the economic potential, life histories, cultural practices, and management of native fishes. Histological analysis helps identify breeding seasons and phenotypic traits, while evaluating the annual breeding cycle of fish for successful cultural practice (Chakraborty *et al.*, 2007; 2010). Therefore, attention should be paid to the negative impact of industrial effluents on the open water fish, *Rasbora daniconius*, for its future existence. In fish, external organs are observed to be affected by toxic

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chemicals, causing loss of balance, increased opercular movement, irregular movement, and ultimately death. Significant damage to internal organs can be the cause. Industrial effluents enter the aquatic ecosystem and make their way into the body of aquatic animals through the gills, digestive tract and general body surface (Pathan, *et al.*, 2010). The effluent accumulates in different tissues of the body. Therefore, it is necessary to carefully study the detailed histopathological alternation or structural changes produced by industrial effluents in different fish organs and study them to assess the extent of damage.

Histopathology is the study of diseased or injured cellular structure. Examination of tissues of fish and other aquatic organisms after death can be used to identify the cause of death and possibly the causative agent (Meyers and Hendricks, 1985). Histology and histopathology could therefore be considered an important tool in the diagnosis, etiology and prevention of diseases (Meyers and Hendricks, 1982).

Over the past three decades, numerous reports indicate that pulp and paper mill effluent have a potentially negative effect on fish reproduction (Munkittrick *et al.*, 1998; Hewitt *et al.*, 2008; and van den Heuvel, 2010). Many studies have reported that paper mill effluent affects the gills, liver, kidneys and ovaries (Khan, 2010; Orrego *et al.*, 2011; Costigan *et al.*, 2012; Pathan *et al.*, 2012; Pathan, 2020).

A review of the literature shows that far fewer studies have examined the effects in men. The present investigation was therefore undertaken with the aim of studying in detail the histopathological changes in the testes of the freshwater fish, *Rasbora daniconius*, after acute and chronic exposure to paper mill effluent.

MATERIAL AND METHODS

Experimental fish: *Rasbora daniconius* were obtained from Godavari River at Kaigaon Toka (latitude 190 37.463 and longitude 750 01.409) 45 km from Aurangabad (MS). The fish were kept in glass aquariums, acclimated for a period of four weeks. During the acclimation period, fish were fed pieces of live earthworms every 24 hours. Healthy fish showing active movements were only considered for the experiment.

Paper Mill Effluent: The paper mill effluent was collected directly from the Kaigaon paper mill at the disposal site located 45 km from Aurangabad. The percentage concentration of the test solution is obtained using the formula (FAO, 1984) which is as follows:

$$\text{Volume percent} = \frac{\text{Volume of effluent}}{V_E + V_{DW}} \times 100$$

VE = Vol. of Effluent, VDW = Vol. of dilution water.

Determination of lethal (LC_{50}) and sublethal concentrations: The 96-hour LC_{50} value was determined by a renewal bioassay after probit analysis (Finney, 1971), due to its advantage over other bioassay techniques. Based on the 96-hour LC_{50} of paper mill effluent, two sublethal concentrations, i.e. concentrations of 1.9% and 0.95% (1/5 and 1/10 of the 96-hour LC_{50} values), were selected for the present study.

Histological biomarkers: Rasbora daniconius (length 8 to 8.5 cm and weight 4 to 4.5 g) was exposed to lethal concentrations (96 hour LC_{50}) of paper mill effluent at a concentration of 9 .5% for 96 hours and at sublethal concentrations of paper mill effluent (1.9% and 0.95%) for 30 days. At the end of the exposure period, surviving fish were sacrificed, carefully dissected to isolate the gills, and fixed in Bouin's fluid. After 24 hours, they were processed using standard technique. Tissues were embedded in paraffin wax and serial sections of 4–6 µm thickness were cut, deparaffinized and stained with hematoxylin and counterstained with eosin. Sections were examined under a light microscope, using (Mcleay, 1987) as a reference, and photographed using a digital camera.

RESULTS AND DISCUSSION

Physicochemical parameters: The values of the physicochemical parameters were observed and recorded in Table 1. The values of the physicochemical parameters of the paper mill effluent are within acceptable limits, except for the values of dissolved oxygen (DO), demand chemical oxygen (COD), oxygen demand (BOD) and hardness. High values of physicochemical parameters may imply increase toxicity.

Histological biomarkers

Testis histology (control): Rasbora daniconius has paired elongated testes that remain structurally suspended from the mesorchium. Each testicle is surrounded by a thick tunica albuginea consisting of a large number of seminiferous tubules tightly linked together by a thin layer of connective tissue. The tubules are of different sizes and very convoluted, separated from each other by a thin stroma of connective tissue and connected to the sperm lumen of the duct lined by a secretary epithelium. The space between the tubules is filled with connective tissue, blood capillaries and interstitial cells (Fig. 1).

Histopathology of testes: Marked degenerative changes in the testes of *Rasbora daniconius* were observed during short- and long-term

exposure to paper mill effluents. Short-term exposure (concentration of 9.5% of paper mill effluent) showed apparent degeneration of the lobules.

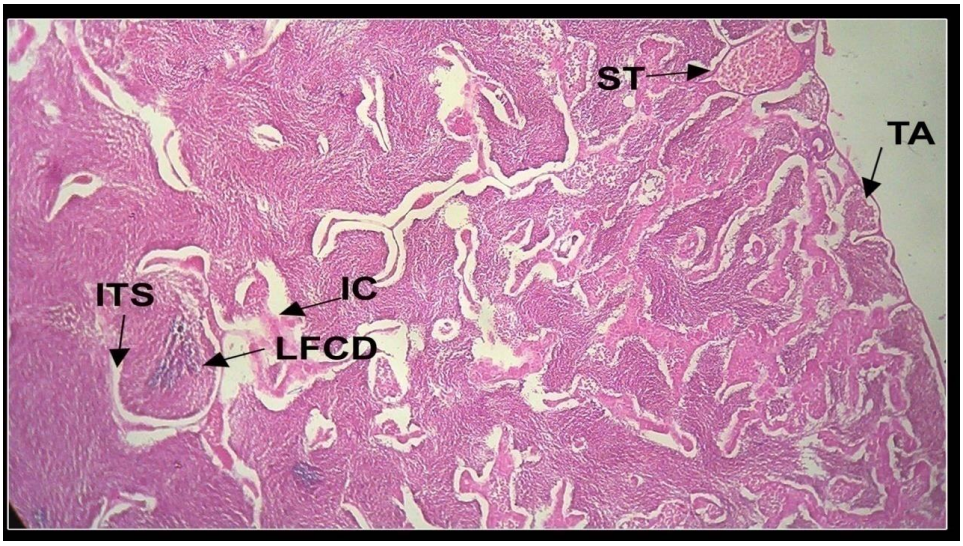

Fig.1. (100x) Transverse section of the testis of control fish, *Rasbora daniconius* showing seminiferous tubules (ST), Lobule Filled with cyst of different development stages (LFCD), Interstitial cells (IC). Tunica albuginea (TA) and Inter-tubular space (ITS)

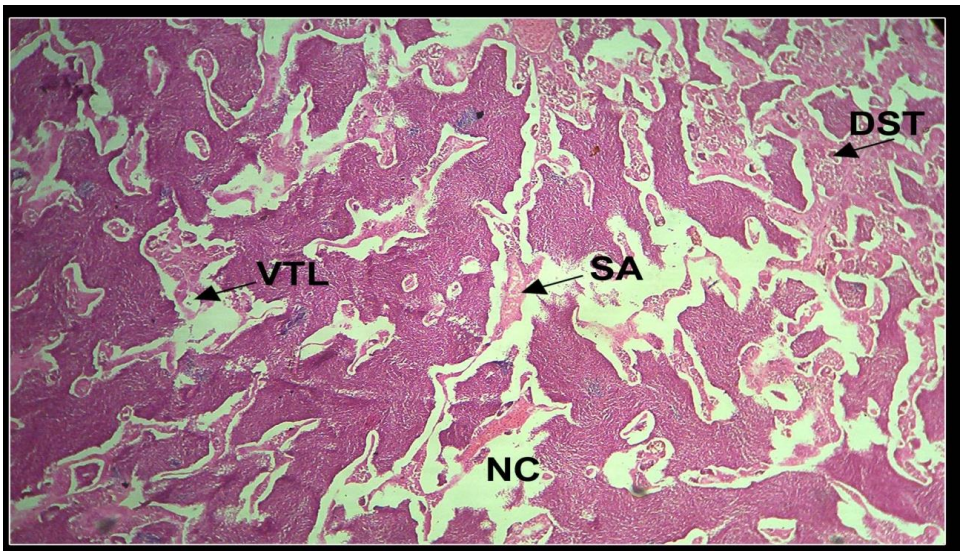

Fig. 2. (100x) after exposure lethal concentration at 9.5% (LC50 of 96 hrs) of paper mill effluent, testis showing Destructed seminiferous tubules (DST), Vacuolated testicular lumen (VTL), Necrosis (NC) and Sperm absorptive stage (SA)

There was necrosis and vacuolation in the testicular lumen, as well as connective tissue (Fig. 2). At 0.95% of paper mill effluent (30-day exposure), fish testes showed significant changes in seminiferous tubules; necrosis and vacuole of the testicular lumen, rupture of tubule

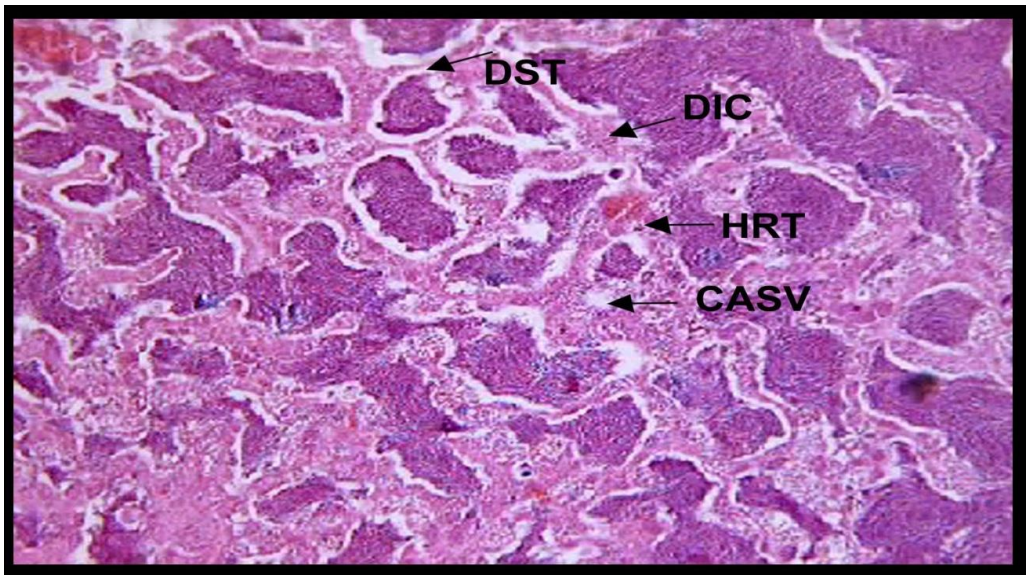

Fig. 3. (400x) after exposure to sublethal concentration at 1.9 % (1/5) of paper mill effluent for 30 days, testis showing Destructed seminiferous tubules (DST) , Disintegrated interstitial cells (DIC), Hemorrhages at the tubules (HRT) and Complete absorption of sperm with cell vacuolation(CASV).

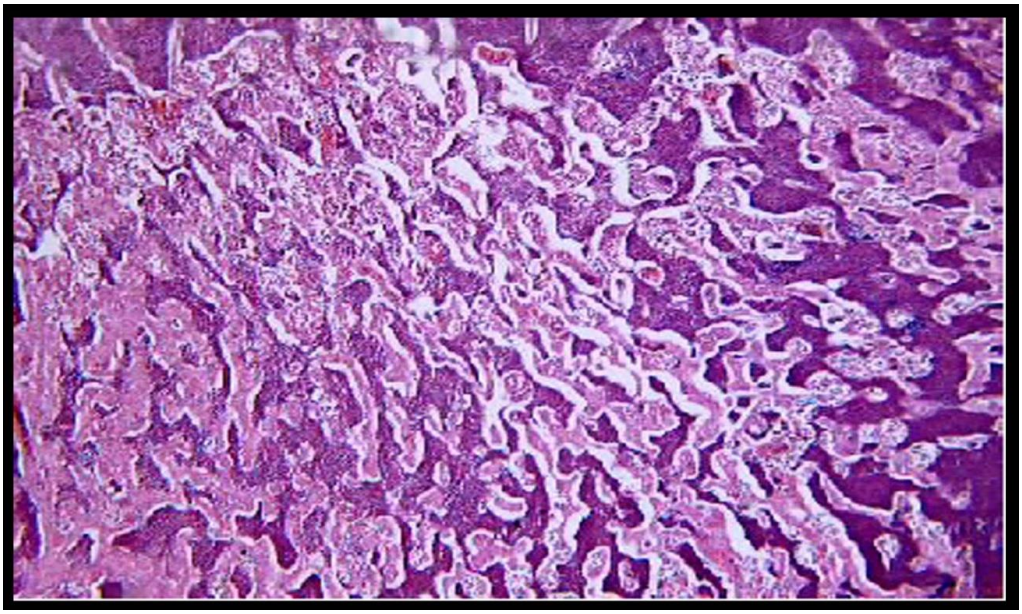

Fig. 4. (100x) after exposure to sublethal concentration at 0.95% (1/10) of paper mill effluent for 30 days, testis showing Destructed seminiferous tubules (DST), Sperm absorptive stage (SA), Hypertrophy of interstitial cells (HIC), Vacuolated testicular lumen (VTL) and Necrosis (NC).

boundaries, cellular hypertrophy and sperm absorption are recorded (Fig. 3).At a sublethal concentration (1.9%) of paper mill effluent for 30,

there were distinct lesions that appeared to break the boundaries of the tubules, liquefaction at the spermatogonium stage, and blood hemorrhage in several locations. Complete sperm absorption with cellular vacuolation was also observed. The interstitial cells were found to disintegrate (Fig. 4). Testis morphology is widely used to detect impacts of anthropogenic chemicals on fish. Very few studies have examined the impacts of pulp mill effluent on testis morphology, as most of these studies assessed effects on testis size (GSI) or secondary sexual characteristics (Hewitt *et al.*, 2008). A study of wild sucker (*Catostomus commersonii*) collected from reference sites and exhibited in pulp mills found no differences in the proportions of spermatogonia, spermatocytes, spermatids, and spermatozoa in the testes (Parrott *et al.*, 2010). Other studies on the mosquito (*Gambusia affinis*) reported no histological abnormalities in testes collected from exposed reference sites or pulp mills from the Dengcun River in China (Hou *et al.*, 2011). Sehgal *et al.* (1984) studied the comparative effect of heavy metal salts on the testicles of Lebistes reticles and observed significant changes after 30 days of exposure; a reduction in the number of different spermatogenic cysts was observed. $CdCl_2$ strongly affected spermatogonial nest cells and spermatocytes, while CuSo4 affected spermatid and sperm cysts. They also observed vacuolation of spermatocytes in guppies treated with $CdCl_2$. with pyknotic spermatocyte nuclei and increased atretic spermatophores in both heavy metals. Staicus (2007) observed a decrease in the number of spermatozoa in the testes of *Carassius auratus gibelio* due to deltamethrin toxicity. Mochida *et al.* (2008) reported a significant reduction in the proliferation of spermatogenic cells and a significant increase in the number of apoptotic germ cells in the testes of *Fundulus heteroclitus* after exposure to tributyltin oxide. Male fish, *Siganus rivalatus*, exposed to industrial effluents showed necrosis, degeneration of mature spermatozoa and inhibition of spermatogenesis with large numbers of spermatids and mature spermatozoa. Fish from mixed effluent showed inhibition of development from early stages of male germ cells to older cells. While necrosis of fish exposed to domestic effluent showed a delay in the conversion of spermatids into spermatozoa and thickening of interlobular tissue was observed by Wahbi and El-Greisry (2007).

In the present investigation, the testes of *Rasbora daniconius* showed marked degenerative changes like degeneration of lobule, absorption of spermatozoa, rupture of tubule boundaries, liquefaction at spermatogonium stage with blood hemorrhage, disintegrated interstitial cells, necrosis and vacuolation in the testicular lumen. The above result

correlates to some extent with the findings of Khillare, 1989; Patil and Dhand, 2000; Sakthivel and Gaikwad, 2001.

CONCLUSION

The present study reveals that paper mill effluents are highly toxic to freshwater fish, *Rasbora daniconius*, exhibiting structural alterations in fish tissues. This parameter could indeed be used as a potential biomarker of toxicity for freshwater fish in the field of environmental biomonitoring.

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