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HISTOPATHOLOGICAL EFFECTS OF PAPER MILL EFFLUENT IN THE OVARY OF A FRESH WATER FISH, RASBORA DANICONIUS

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Abstract- The main objective of the present investigation was to study the histopathological effects of paper mill effluent in the ovary of fresh-water fish, *Rasbora daniconius.* In this experiment, we exposed the fish, *Rasbora daniconius* to lethal concentration at 9.5% (LC_{50} of 96 hrs) for 96 hrs and sublethal concentrations [1.9 % (1/5) and 0.95 % (1/10) LC_{50} of 96hrs] for 30 days. A second group of fish without exposure to paper mill effluent served as the control. The control ovaries appeared structurally normal. Ovaries of fish exposed to lethal concentration for 96 hr at 9.5% (LC_{50}) of paper mill effluent showed different types of arteria in mature follicles, complete absorption of oocytes. Most of zona radiata are segmented and broken down in several sites. At 0.95% concentration of paper mill effluent for 30 days exposure showed thick ovarian wall, cytoplasmic clumping, cytoplasmic liquification and disappearance of nucleus were observed in mature oocytes. After 1.9% concentration exposure of paper mill effluent for 30 days, shows damaged oocytes, thickening of ovarian wall; cytoplasmic liquification and disappearance of nucleus in mature oocytes.
Key words: *Rasbora daniconius,* paper mill effluent, ovary, and histopathology.

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Introduction

The pulp and paper mill is the sixth largest polluting industry after oil, cement, leather, textile and steel industries, as it discharges a variety of gaseous, liquid and solid waste in to the environment [1]. The major concerns are with the ecotoxicological impacts in the receiving water bodies because large volume of wastewater are generated by pulp and paper mills and freshwater, estuarine, and marine ecosystem affecting aquatic biota and having negative on human health [2,3].

Due to high chemical diversity of the organic pollutants in paper and pulp mill effluent, a high variety of toxic effects on aquatic communities in recipient water courses have been observed [4,5]. A significant number of these substances have been classified as carcinogenic, mutagenic and clastogenic [6,7] and endocrinic [8]. The pollutants concerned also kill fish or affect their reproductive physiology [9], or may induce male-biased sex ratios among fish embryos [10].

Histopathological changes have been used as biomarkers in the evaluation of the health of fish exposed contaminants, both in the laboratory [11,12] and field studies [13,14,15].

Very few studies have been carried out in connection with the histopathological effects of pulp and paper mill effluent in fish. [16] reported the occurrence of fin necrosis, kidney tumors, anemia change in parastiofauna, low condition factors and organ somatic indices of winter flounder living in the vicinity of a pulp and paper mill. Discharge of untreated pulp and paper effluent into receiving waters is known to be toxic to some aquatic organisms. Manifestations of toxicity in fish includes fin necrosis, increase of parasites, changes in physiology, detoxifying enzyme activities, hematology, osmoregulation and reproduction [18,19,20,21]. Lesions have also observed the gill and liver of effluent Exposed fish [22]. However, scientific evidence of toxicological impact of paper mill effluent on the reproductive system is completely lacking. The focus of the present study was therefore; to investigate the paper mill effluent

induced ovarian histopathology of a freshwater fish, Rasbora daniconius both on acute and chronic exposure.

Experimental

Experimental fish

The Rasbora daniconius were obtained from Godavari river at Kaigaon Toka (latitude 190 37.463 and longitude 750 01.409) 45 km away from Aurangabad (MS). The fishes were kept in glass aquaria, acclimatized for the period of four weeks. During period of acclimatization the fishes were fed after every 24 hours on pieces of live earthworms. Healthy fishes showed active movement were only considered for the experimentation.

Paper mill effluent

The Paper mill effluent was collected directly from the Kaigaon paper mill at releasing site 45 Km away from Aurangabad. The percentage concentration of test solution is obtained by using formula [23], which is as follow.

V_E = Vol. of Effluent, V_{DW} = Vol. of Dilution water.

Determination of lethal (LC_{50}) and sublethal concentrations

The LC_{50} value for 96 hrs was determined by renewal bioassay following probit analysis [24], due to its advantage over other bioassay techniques. This method has advantage of replacing the toxicant solution a fresh every 24h so that metabolic waste (ammonia)

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

which itself highly toxic can be removed. The LC_{50} values for the 96 hr period of paper mill effluent was found to be 9.5% concentration and 1.9% and 0.95% concentration (1/5 and 1/10 of the LC_{50} values for 96 hrs) was selected as the sublethal concentrations for chronic studies.

Histological Biomarkers

Rasbora daniconius (length 8 to 8.5 cm and weight 4 to 4.5 gm) were exposed to lethal (96 hrs LC_{50}) concentration of paper mill effluent at 9.5% concentration for 96s hr and sublethal concentrations of paper mill effluent (1.9% and 0.95%) for 30 days. At the end of exposure period the fishes survived were sacrificed dissect carefully to isolate gill and fixed in bounis fluid. After 24 hrs they were processed following the standards technique. Tissues were embedded in paraffin wax and serial section of 4-6 µ m thickness were cut, deparaffinised and stained in haematoxylin and counterstained with eosin. The sections were examined under light microscopy, using [25] as a reference, and photographed using a digital camera.

Results and Discussion

Histopathology of Ovary

The ovaries fish from control group showed normal histological structure (Figure 1). The Ovaries of fish exposed to lethal concentration for 96 hr at 9.5% (LC_{50}) of paper mill effluent showed different types of arteria that are common in mature follicles and complete absorption of oocytes. Most of zona radiata are segmented and broken down in several sites. Degeneration of Some oocytes is noticed. In oocytes of treated fish nucleus exhibited degeneration which is evidence initially by the liquification of the prenuclear cytoplasm (Figure 2). Chronic exposure showed significant changes in the histological structures of ovary. After 1.9% concentration exposure of paper mill effluent for 30 days, it was observed that mature oocytes found more damaged than the primary oocytes. At several places artertic condition and absorptions of growing oocytes are observed. Thickening of ovarian wall was noticed. Cytoplasmic liquification, wall rupture and disappearance of nucleus were observed in mature oocytes (Figure 3). At 0.95% concentration of paper mill effluent for 30 days exposure showed thick ovarian wall, cytoplasmic clumping, cytoplasmic liquification and disappearance of nucleus were observed in mature oocytes. Most of zona radiata broken down several sites invade the oocytes and devoured the surrounding cytoplasm. Proliferation of follicular theca cells are shown in ovaries (Figure 3).

The impact of untreated effluent discharged by pulp and paper mills hinge not only on the toxic components but also on other variables such as oxygen demand, pH, colour and suspended solids, which singly or collectively might produced a variety of biological effects [26].

Histopathology provides a rapid method to detect effects of irritants in various organs [27]. The exposure of fish to chemical contaminants likely to induces a number of lesions in different organs [28]. [29]observed histopathological changes in the ovaries of *C. fasciatus* when exposed to sublethal concentration of nickel. The prominent changes were occurrence of atretic oocytes and increase in the interfollicular space. [30] exposed *H. fossilis* to textile mill effluent and observed histopathological changes in the ovary. They found nuclei of the oogonia underwent complete karyolysis, vacuolation within nucleoli and shrinkage of nucleus in the ovary of treated fish. [31]observed reduction in diameter of oocytes I, II and III. Prominent interfollicular space was observed in the ovaries which were probably formed due to shrinkage of the oocytes. Large numbers of atretic follicles were also observed in the ovaries of *H. fosillis* exposed to chlordeconc. [32] studied the effect of carbaryl on the ovary of *Clarias batrachus* and observed vacuolation and necrosis, arrested ovarian recrudescence and interfollicular oedema.

[33]noted partial lysis, swelling, atersia and changes in nucleus and cytoplasmic organization after exposure of *Heteropneustes fossilis* to paper mill effluent for 20 days. They suggested that alterations were due to influence on the pituitary gonadal axis. According to [34] during different development phases of ovary, tannery effluent caused profound changes and he concluded that tannery effluent containing variety of chemical and metal affect the ovarian development at different stage. [35] reported that the ovaries of female medaka, *Oryzias latipes* from both equol and genistein treatment showed delayed oocytes maturation, atretic oocytes, enlargement lumen, proliferation of somatic stroma tissue and primordial germ cells. They also stated that the responses were concentrations dependent. [36] identified the rupturing of oocytes membrane in the oocytes, vacuolization in the peripheral oocytes and disturbances in the supporting connective tissue after acute and chronic exposure of sugar industrial effluent to Crab, *Barytelphusa guerini.* [37] observed degenerative changes in the ovary when exposed to $HgCl_2$ and $CdCl_2$ and its combination.

In the present investigation, ovary of *Rasbora daniconius* subjected to paper mill effluent showed marked degenerative changes like complete absorption of oocytes, broken zona radiata, degeneration of oocytes, cytoplasmic liquification and clumping, thickening of ovarian wall and disappearance of nucleus were observed in mature oocytes. The above results correlate with the findings of

[37,38] Ramachandra Mohan [2000], Wahbi and El-Greisry [2007]. Ovarian changes noted in the present study can be attributed to either direct action of paper mill effluent on the ovary.

Fig. 1- (400x) transverse section of the ovary of control fish, Rasbora daniconius showing primary oocyte (PO), Matured ova (MO), Growing Follicle (GF), Secondary oocyte (SO), Germinal epithelium (GE).

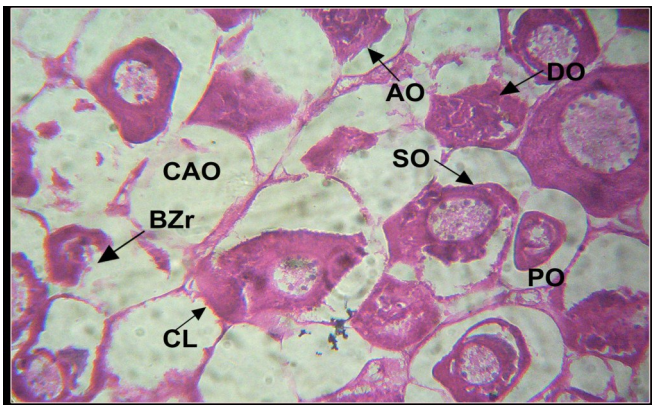

Fig. 2- (400x) after exposure lethal concentration at 9.5% (LC50 of 96 hrs) of paper mill effluent, ovary showing primary oocyte (PO), Secondary oocyte (SO), Complete absorption oocyte (CAO), Atretic condition of oocyte (AO), Cytoplasmic liquification (CL), Broken zona radiata (BZr), Degeneration of oocyte (DO)

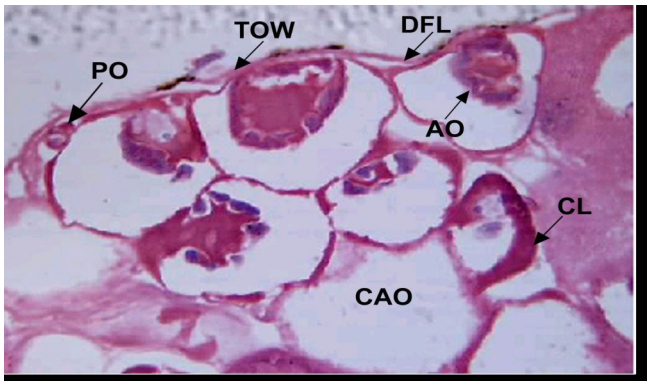

Fig. 3- (400x) after exposure to sublethal concentration at 1.9 % (1/5) of paper mill effluent for 30 days, gill showing primary oocyte (PO), Complete absorption oocyte (CAO), Atretic condition of oocyte (AO), Cytoplasmic liquification (CL), Detachment of follicle from lamellae (DFL), Thickening of Ovarian wall (TOW).

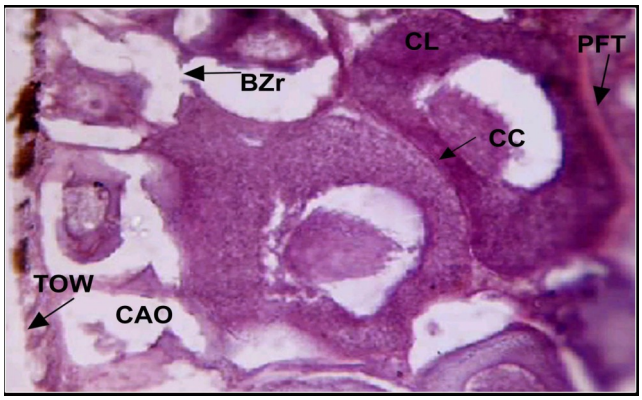

Fig. 4- (400x) after exposure to sublethal concentration at 0.95% (1/10) of paper mill effluent for 30 days, gill showing Proliferation of follicular theca wall (PFT), Complete absorption oocyte (CAO), Cytoplasmic clumping (CC), Cytoplasmic liquification (CL), Broken zona radiata (BZr), Thickening of Ovarian wall (TOW).

The results obtained during this study, it can be concluded that the ovary histology of *Rasbora daniconius* appears to be sensitive monitoring tool to aquatic health. Fish histopathology could therefore make valuable contribution in the monitoring of aquatic ecosystems and should form an important part of environmental impact assessment in the environmental management process.

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