



HISTOPATHOLOGICAL ALTERATIONS IN THE BLOOD OF *Oreochromis mossambicus* EXPOSED TO PULP AND PAPERMILL EFFLUENT

KIRAN JOSEPH ^{a#*} AND ELIZABETH JOHN ^{b#}

^a Department of Zoology, Catholicate College, Pathanamthitta, India.

^b Department of Zoology, St. Gregorios College, Kottarakkara, India.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Toxicological effect of pulp and papermill effluent in the fish, *Oreochromis mossambicus* was investigated in the present study. Pulp and papermill effluent discharged from Hindustan News Print LTD, Peruva, Kottayam, Kerala were collected and adult fish of size 15 ± 2 g were exposed to a sublethal concentrations (1/5th and 1/10th LC50 value) of the effluent for a period of 10, 20 and 30 days. The treated fish were compared with the control group for the histological alterations in the blood smear. The study revealed marked changes in the blood tissues of the effluent treated fish.

Keywords: *Oreochromis mossambicus*; histopathology; pulp and papermill effluent; blood smear.

1. INTRODUCTION

Pollution on freshwater surfaces is a major environmental problem worldwide. Deterioration in these environments due to human activities has created a need for developing and adjusting methods for environmental quality. Due to urban, industrial and agricultural activities, freshwater sources are dumped with different kinds of chemicals that affect the inhabiting biota. In order to evaluate the adverse

effects of these complex chemical mixtures on aquatic organism, there is a worldwide trend to complement chemical and physical parameters with biomarkers in aquatic pollution monitoring [1,2].

The use of histopathological markers has already been tested and proposed as an efficient and sensitive method to monitor fish health and environmental pollution in natural water bodies [3,4,5]. Such biomarkers might indicate acute or chronic exposure

[#] Assistant Professor;

^{*} Corresponding author: Email: kirankj007@gmail.com;

to contaminants and facilitate the detection of fish physiological responses, thereby establishing a more realistic diagnosis for evaluating environmental health [6].

Histopathological biomarkers are closely related to other biomarkers of stress since many pollutants have to undergo metabolic activation in order to be able to provoke cellular change in the affected organism [7]. Histopathological investigations may prove not only to be a biomarker of prior exposure to toxicants, but also a cost-effective tool to determine the health of fish populations, hence reflecting the health of the entire freshwater ecosystem. Therefore, it is necessary to study the detail histopathological alternations or changes in structure produced by industrial effluent in different organs of fishes thoroughly investigate them in order to assess the extent of damage.

Temporal or spatial changes in baseline haematology and histology of blood cells may provide a specific indication of changes within the environment of the test species. The blood is one of the circulating media of the body and entry of any toxicant can be detected by analyzing the blood [8]. Blood is an important tool in diagnosing the structural and functional status of fish exposed to toxicants [9]. The most frequently observed changes in blood cells are anisocytosis, poikilocytosis, pyknosis, necrosis, rouleux formation etc., [10].

2. MATERIALS AND METHODS

Effluent sample was collected from Hindustan News Print Ltd. effluent tank at Velloor, Kottayam (Dist), Kerala, India. The pH noticed in the effluent was 8.5. The measured values for total dissolved solids, total hardness, total alkalinity and dissolved oxygen were 2900 mg/l, 560 mg/l, 165 mg/l and 1.52 mg/l respectively. The pH noticed for the dilution water was 7.0. The measured values for total dissolved solids, total hardness, total alkalinity and dissolved oxygen were 121mg/l, 27mg/l, 7.2mg/l and 8.1mg/l respectively.

Mature male and female *Oreochromis mossambicus* were collected from Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Aliyar, Tamil Nadu, India. Fishes were stored in the laboratory in four general holding tanks (glass aquaria) of 500 liters capacity. In the general holding tanks the fish were acclimatized for 15 days with a light and dark photoperiod of 12hr/12hr and the temperature of water was $25\pm 2^{\circ}\text{C}$. The room temperature of the laboratory was held constant at

$27\pm 2^{\circ}\text{C}$. During acclimatization period both males and females were kept in separate tanks. The fishes were fed daily with commercial fish feed.

2.1 Sublethal Toxicity Study

Based on probit analysis, regression equation for Pulp and papermill effluent (96 h exposure) was $Y = 1.814x + 45.75$. The LC_{50} value of the Pulp and papermill effluent to *Oreochromis mossambicus* was 2.73 %. Sublethal concentration of the test was made at $1/5^{\text{th}}$ (0.546%) and $1/10^{\text{th}}$ (0.273%) of LC_{50} value.

Ninety healthy fishes were selected from the stock for sublethal exposure study. It comprised of 45 male and 45 female. Six experimental groups and one control group were considered. Each group consisted of 5 male and 5 female fishes of size 9-12cm and weighed 15 – 20gm. 10, 20 and 30 days of exposure periods were undertaken for each concentration. After the end of each exposure period four healthy male and four healthy female were sacrificed immediately after taking a drop of blood from caudal vein for blood smear preparation.

2.2 Staining and Mounting

Two histological stains were employed and included a routine Haematoxylin and Eosin (H&E) stain. The procedure followed for each of these stains was according to the adapted methods as listed by Van Dyk [11]. Once stained, all slides were mounted with Canada balsam

3. RESULTS

The fish considered for control showed normal histological structure of different blood components. The peripheral blood smear of fish under control showed normal structure and number of red blood corpuscles (RBC), white blood corpuscles (WBC) and lymphocytes (L) (Phm:1).

In experimental fishes the histological assessment of peripheral blood smear showed abnormalities in the structural view of different types of blood cells (Phm:2-7). The abnormal conditions such as, increase in the number of WBC, anisocytosis (AC) and poikilocytosis (PC) were noticed in both concentrations of paper mill effluent exposed fish groups for 10, 20 and 30 days. Presence of double nuclei (DN) was observed in $1/10^{\text{th}}$ and $1/5^{\text{th}}$ concentrations of effluent exposed groups for 20 and 30 days. Roulex formation (RF) was observed in $1/5^{\text{th}}$ concentration in 30 days exposed fish.

4. DISCUSSION

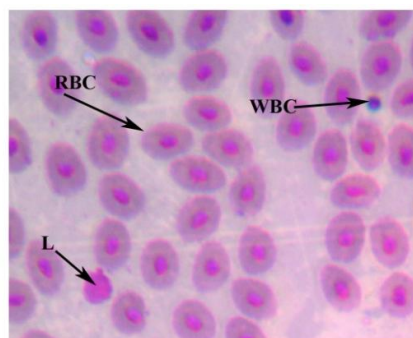
Histological observation studies on the peripheral blood tissue has gained utmost importance in determining the effects of environmental pollution. It was noticed that the structure of red blood cells of fish exposed to paper mill effluent showed deformation. Due to toxicity, the morphology of red blood cells changed. Similar observations were made by Jeney et al. [12] in Roach on exposure to paper mill effluent.

Disorders in the nucleus i.e., presence of double nuclei was noticed in the effluent treated fish. Presence of double nuclei (one micronuclei and another normal) is not definitively known what effect these anomalies have on the organism. Some authors have suggested that direct nuclear division without mitotic apparatus is induced by unfavorable factors and that any type of nuclear change in the red blood cells is a sign of cell degeneration and necrosis [13,14].

Increase in the number of white blood cells was another deformity noticed in the effluent treated fish. This may attributed to the increased defensive mechanism exhibited in the effluent exposure. In

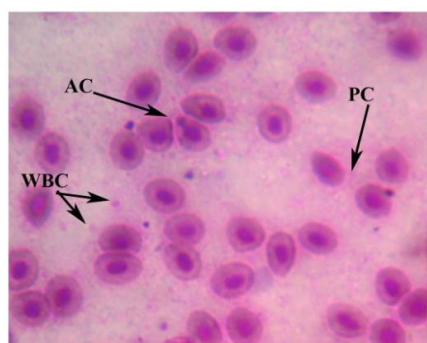
exposed fishes during exposure period, poikilocytosis in the erythrocytes, and anisocytosis was observed. The presence of abnormality in the shape and size of erythrocytes (poikilocytosis and anisocytosis) were noticed in the study was in associated with an altered surface membrane area to volume ratio. The deformation of erythrocytes was obvious, and has possibly reduced the capacity of oxygen transport, consequently causing a certain level of hypoxia. Similar conditions of haematopathology were reported in *Oreochromis mossambicus* on exposure to biocide chlorine (NaOCl) [15]. Such deformities were noticed in fresh water teleost on exposure to mercuric chloride [16,17].

Moreover, in the peripheral blood of the fish samples taken from fish exposed to higher concentration at 30 days exposure showed rouleux formation. The histological abnormalities such as rouleux formation of erythrocytes of the effluent exposed fishes, could be attributed to the cytoskeleton fragility and defects induced by the toxicant. Similar observations were made by Ranu et al. and Kamleshwar et al [18,19] in *Clarias batrachus* on exposure to aluminium.



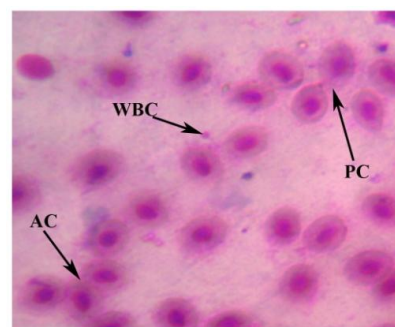
Phm 1. Blood smear control HE .50µm

RBC- Red blood corpuscles
WBC- White blood corpuscles
L- Lymphocyte



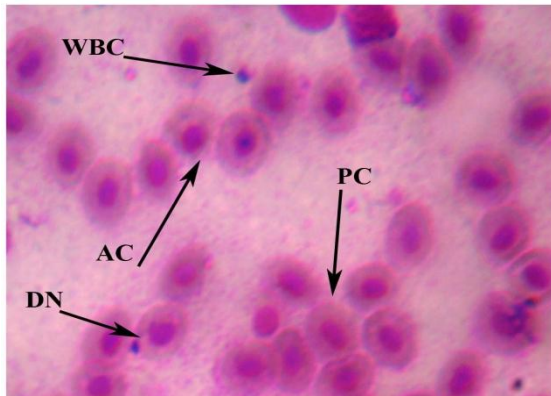
Phm 2. Blood smear 1/10th Conc. 10days HE .50 µm

AC- Anisocytosis
PC-Poikilocytosis
WBC- White blood Corpuscles

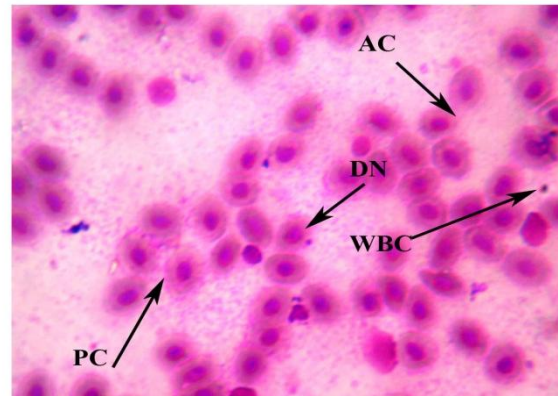


Phm 3. Blood smear 1/5th Conc. 10days HE .50 µm

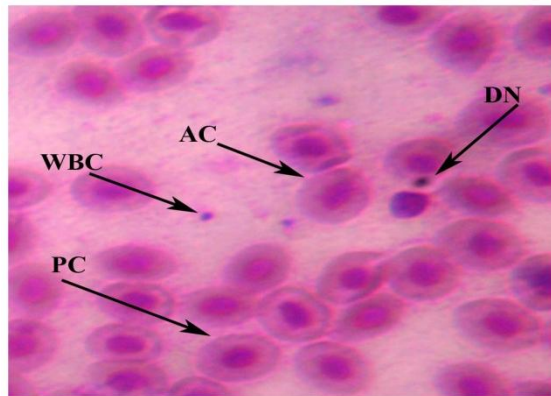
AC- Anisocytosis
PC-Poikilocytosis
WBC- White blood Corpuscles



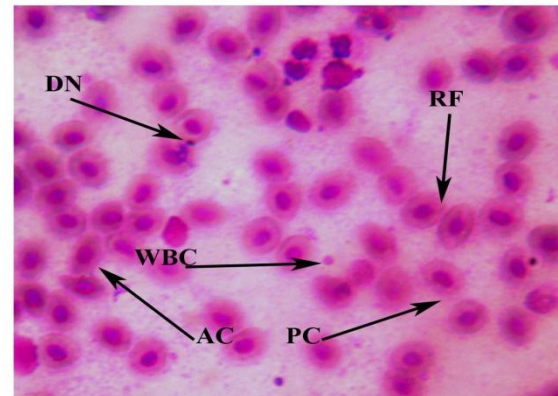
Phm 4. Blood smear 1/10th Conc. 20days HE .50 µm
AC- Anisocytosis
PC-Poikilocytosis
WBC- White blood Corpuscles
DN-Double nuclei



Phm 5. Blood smear 1/5th Conc. 20days HE .50 µm
AC- Anisocytosis
PC-Poikilocytosis
WBC- White blood Corpuscles
DN-Double nuclei



Phm 6. Blood smear 1/10th Conc. 30days HE .50 µm
AC- Anisocytosis
PC-Poikilocytosis
WBC- White blood Corpuscles
DN-Double nuclei



Phm 7. Blood smear 1/5th Conc. 30days HE .50 µm
AC- Anisocytosis
PC-Poikilocytosis
RF-Rouleux formation
WBC- White blood Corpuscles
DN-Double nuclei

5. CONCLUSION

Histological assessment of fish exposed to pulp and papermill effluent revealed remarkable alterations in the blood. In experimental fishes the histological assessment of peripheral blood smear showed abnormalities in the structural view of different types of blood cells. The abnormal conditions such as, increase in the number of WBC, anisocytosis (AC) and poikilocytosis (PC) were noticed in both concentrations of paper mill effluent exposed fish groups for 10, 20 and 30 days. Roulex formation (RF) was observed in 1/5th concentration in 30 days exposed fish.

COMPETING INTERESTS

I hereby declared that no competing interests exist. This is an original research work done by me. The

products used for this research are commonly and predominantly use products in our area of research and country. Also, the research was not funded by any agency and this work funded by my personal efforts.

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