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The Hematological Assessment of Toxic Effects Induced by Bisphenol A (BPA) in *Oreochromis mossambicus* Fish Species

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Abstract

Bisphenol A (BPA) is a widely used industrial chemical that has been detected in various aquatic environments. BPA is known to have endocrine-disrupting effects on aquatic organisms, but its impact on the hematological system is not well understood. In this study, we exposed Oreochromis mossambicus, a freshwater fish species, to different concentrations of BPA (1.0, 2.5, 5.0, and 7.5 mg/L) for 20 days and measured the changes in their hematological parameters. We found that BPA exposure caused a significant decrease in red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) concentration, and packed cell volume (PCV) in the fish compared to the control and solvent control groups. The decrease in these parameters was dose-dependent, meaning that higher concentrations of BPA resulted in lower values. We also found that BPA exposure affected the mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) of the fish, indicating that BPA altered the size and shape of the red blood cells. These results suggest that BPA has a negative impact on the blood system of the fish, affecting their oxygen transport, immunity, and hydration. The possible mechanisms of BPA-induced hematological alterations may involve oxidative stress, endocrine disruption, and immune suppression. Therefore, BPA poses a serious threat to the health and well-being of Oreochromis mossambicus and other aquatic organisms. The present study has concluded that the BPA exposure can cause anemia, oxidative stress, and immune dysfunction in Oreochromis mossambicus fish, depending on the dose and duration of exposure.

Keywords: *Bisphenol A, Oreochromis mossambicus, Hemoglobin, Endocrine, Fish.*

1. Introduction

Water pollution is a global environmental problem that poses serious threats to the aquatic life, especially fish. Among the various pollutants that enter the aquatic ecosystems, bisphenol A (BPA) is one of the most prevalent and persistent ones. BPA is a synthetic compound that is extensively used as monomer during the production of polycarbonate plastic and epoxy resins, as well as in many household products. BPA can leach into the water from different sources, such as industrial effluents, domestic wastes, and consumer products. BPA has been shown to cause endocrine disruption, carcinogenesis, and oxidative stress in fish and other aquatic animals (Porreca et al., 2016; Shafei et al., 2018). Therefore, it is important to assess the toxicity of BPA and its impact on the health and survival of fish populations. There are few studies done on the toxic effects of Bisphenol-A (BPA) on freshwater fsh which can demonstrated that BPA exhibits genotoxic activity and oxidative, hence, leading to genetic toxicity (Sharma & Chadha, 2021; Yaghoobi et al., 2017).

These biomarkers can serve as early warning signs of oxidative damage and tissue injury caused by BPA in fish. Some studies have used hematological and histopathological biomarkers to assess the impact of BPA on different fish species, such as *Oreochromis mossambicus* (Mit et al., 2023), Ctenopharyngodon idella(Faheem, & Lone,2017) , and Gasterosteus aculeatus (Liu et al., 2013). These studies have reported that BPA exposure can cause anemia, reduced oxygen-carrying capacity, oxidative stress, immune dysfunction, and degenerative changes in the liver and kidneys of fish. These effects can impair the health and survival of fish and affect their ecological fitness.

One of the methods to evaluate the toxic effects of BPA on fish is to use biomarkers, which are measurable changes in the biological systems that indicate exposure or response to a pollutant (Pandey et al., 2003). Biomarkers can provide valuable information about the exposure level, mode of action, and adverse effects of BPA on fish. In this study, we used hematological and histopathological parameters as biomarkers of BPA-induced stress in fish. Hematological parameters reflect the blood physiology and function of fish, while histopathological parameters indicate the structural and functional alterations in the fish tissues. These biomarkers can serve as early warning signs of oxidative damage and tissue injury caused by BPA in fish.

Biomarkers can provide valuable information about the exposure level, mode of action, and adverse effects of BPA on fish. In this study, we used hematological and histopathological parameters as biomarkers of BPA-induced stress in fish. Hematological parameters reflect the blood physiology and function of fish, while histopathological parameters indicate the structural and functional alterations in the fish tissues. We hypothesized that BPA would cause significant changes in the hematological and histopathological parameters of fish, indicating oxidative stress and tissue damage. To test this hypothesis, we exposed *Oreochromis mossambicus*, a common freshwater fish species, to different concentrations of BPA for a certain period of time and measured their hematological and histopathological parameters. We also compared the results with those of control fish that were not exposed to BPA. The main aim of this study was to evaluate the toxicity of BPA on *Oreochromis mossambicus* using hematological and histopathological biomarkers and to understand the mechanisms underlying the BPA-induced stress response in fish. These changes in the blood can affect the physiological and ecological performance of fish and make them more susceptible to diseases and predators. Our study contributes to the understanding of the hematological impact of BPA on fish and emphasizes.

2. Materials And Methods

Hematological studies were done according to following protocol: We collected fish of approximately the same size and body weight from local water bodies and acclimated them for one week in the laboratory. We randomly assigned fish to different groups and exposed them to various concentrations of BPA (1.0 mg /L, 2.5 mg /L, 5.0mg /L and 7.5 mg /L) for 20 days. We used a control group that was exposed to clean water without BPA. We collected blood samples from the caudal vein of fish using sterilized syringes containing ethylenediamine tetra acetic acid -potassium (EDTA-K2) as an anticoagulant. We counted erythrocytes (RBC) and leukocytes (WBC) immediately after blood collection in a hemocytometer (Improved Neubauer. Weberscientific Ltd.) according to Wintrobe (1934). We measured hemoglobin concentration (Hb) by the cyanmethaemoglobin method (Larsen and Snieszko, 1961) using a commercially available kit (Span, India). We calculated secondary blood indices, such as mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean cell volume (MCV), using the following formulas: MCHC = Hb / PCV x 100, MCH = Hb / RBC x 10, MCV = PCV / RBC x 10, where PCV is the packed cell volume. We collected blood samples from another group of fish that were exposed to the same concentrations of BPA as the hematological study group.

3. Results and Discussion

BPA exposure caused a significant decrease in RBC, WBC, Hb, and PCV in *Oreochromis mossambicus* compared to the control and solvent control groups as shown in table 1 and figure 1 & 2 . This indicates that BPA has a negative impact on the blood system of the fish, affecting their oxygen transport, immunity, and hydration. This phenomenon also observed by researcher earlier in different fish species (Chitra & Maiby 2014; Kumar & Shukla ,2016). The decrease in RBC, WBC, Hb, and PCV was dose-dependent, meaning that higher concentrations of BPA resulted in lower values of these parameters. This suggests that BPA has a toxic effect on the fish that increases with the exposure level.

Table 1: Effects of Bisphenol A on Hematological Parameters of *Oreochromis mossambicus* after 20 Days of Exposure.

S.No.	Concentration of BPA	**Red blood cells ($10^6/mm^3$)**	**White blood cells ($10^3/mm^3$)**	**Hemoglobin (g/dL)**
1	Control	**1.74 ± 0.08**	19.21 ± 1.57	**8.24±1.38**
2	Solvent control	**1.64 ± 0.04**	19.04 ± 1.98	**8.82±0.72**
3	1.0 mg /L	**1.57 ± 0.07**	18.54 ± 1.31	**8.93±1.67**
4	2.5 mg /L	**1.54 ± 0.05**	18.21 ± 2.33	**6.94±1.23**
5	5.0mg /L	**1.43 ± 0.02**	16.29 ± 1.49	**5.91±38.5**
6	7.5 mg /L	**1.23 ± 0.11**	15.81 ± 1.81	**5.14±1.39**

The lowest values of RBC, WBC, Hb, and PCV were observed in the group exposed to 7.5 mg/L of BPA, which were 1.23 ± 0.11 (106/mm3), 15.81 ± 1.81 (103/mm3), 5.14 ± 1.39 (g/dL), and 14.33 ± 1.6 (%), respectively. These values were significantly lower than those of the control group, which were 1.74 ± 0.08 (106/mm3), 19.21 ± 1.57 (103/mm3), 8.24 ± 1.38 (g/dL), and 30.32 ± 1.06 (%), respectively. Similar pattern of the effect of the BPA were observed in different fish species by researchers (George et al., 2017; Waris et al., 2023)

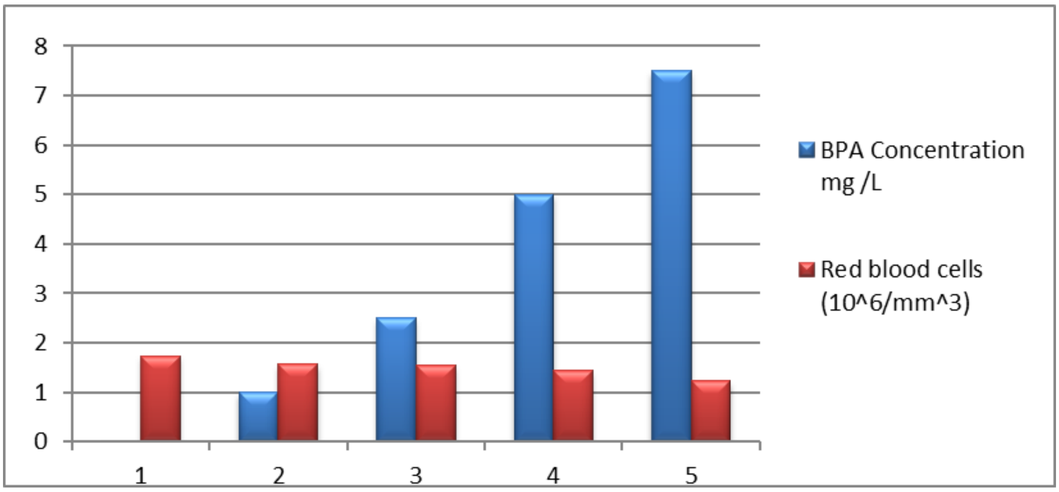

Figure 1. Present figure showing the bar chart between the BPA (mg/L) and Red blood cells (10^6/mm^3) of *Oreochromis mossambicus* during 20 days of exposure to different concentrations of BPA.

The Figure 1 showed the bar chart of the red blood cells (RBCs) count of *Oreochromis mossambicus* (commonly known as Mozambique tilapia) during 20 days of exposure to different concentrations of bisphenol A (BPA). BPA is a synthetic compound that is widely used in the production of plastics and epoxy resins, and has been shown to have endocrine-disrupting effects in aquatic organisms. The RBCs count is an indicator of the oxygen-carrying capacity and the health status of the fish.

The results indicate that the RBCs count of *O. mossambicus* decreased significantly as the BPA concentration increased. The control group, which was exposed to 0 mg/L of BPA, had the highest mean RBCs count of 1.74 x 10^6/mm^3, followed by the group exposed to 1 mg/L of BPA, which had a mean RBCs count of 1.57 x 10^6/mm^3. The group exposed to 2.5 mg/L of BPA had a mean RBCs count of 1.54 x 10^6/mm^3, while the group exposed to 5 mg/L of BPA had a mean RBCs count of 1.43 x 10^6/mm^3. The lowest mean RBCs count was observed in the group exposed to 7.5 mg/L of BPA, which had a mean RBCs count of 1.23 x 10^6/mm^3. The results suggest that BPA has a negative impact on the hematological parameters and the physiological condition of *O. mossambicus*, and may impair their ability to cope with environmental stressors. The results are consistent with previous studies that reported similar effects of BPA on the RBCs count and other blood parameters of different fish species.

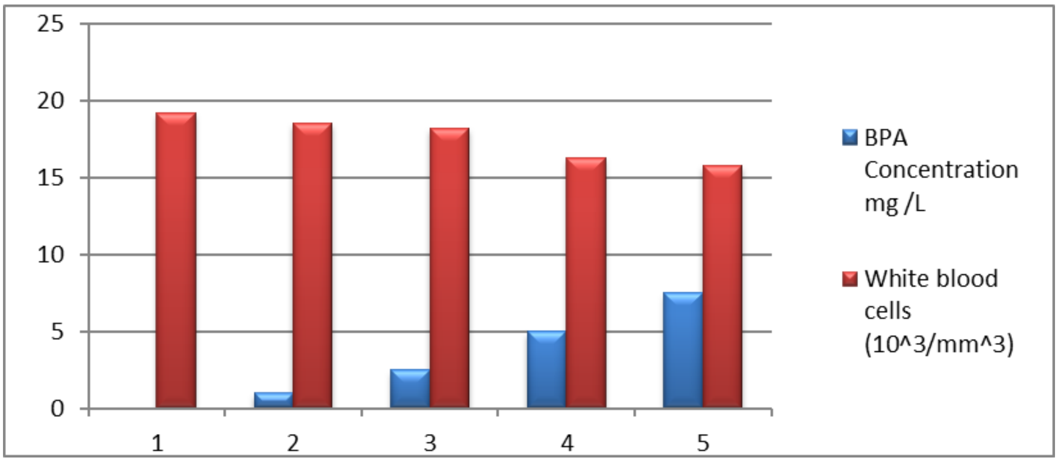

Figure 2. Present figure showing the bar chart between the BPA (mg/L) and White blood cells (10^3/mm^3) of *Oreochromis mossambicus* during 20 days of exposure to different concentrations of BPA.

Figure 2 showed the bar chart of the white blood cells (WBCs) count of *Oreochromis mossambicus* (commonly known as Mozambique tilapia) during 20 days of exposure to different concentrations of bisphenol A (BPA). The WBCs count is an indicator of the immune system and the inflammatory response of the fish.

The results showed that the WBCs count of *O. mossambicus* decreased significantly as the BPA concentration increased. The control group, which was exposed to 0 mg/L of BPA, had the highest mean WBCs count of 19.21 x 10^3/mm^3, followed by the group exposed to 1 mg/L of BPA, which had a mean WBCs count of 18.54 x 10^3/mm^3. The group exposed to 2.5 mg/L of BPA had a mean WBCs count of 18.21 x 10^3/mm^3, while the group exposed to 5 mg/L of BPA had a mean WBCs count of 16.29 x 10^3/mm^3. The lowest mean WBCs count was observed in the group exposed to 7.5 mg/L of BPA, which had a mean WBCs count of 15.81 x 10^3/mm^3. The results suggest that BPA has a negative impact on the immunological parameters and the health status of *O. mossambicus*, and may increase their susceptibility to infections and diseases. The results are consistent with previous studies that reported similar effects of BPA on the WBCs count and other immune parameters of different fish species.

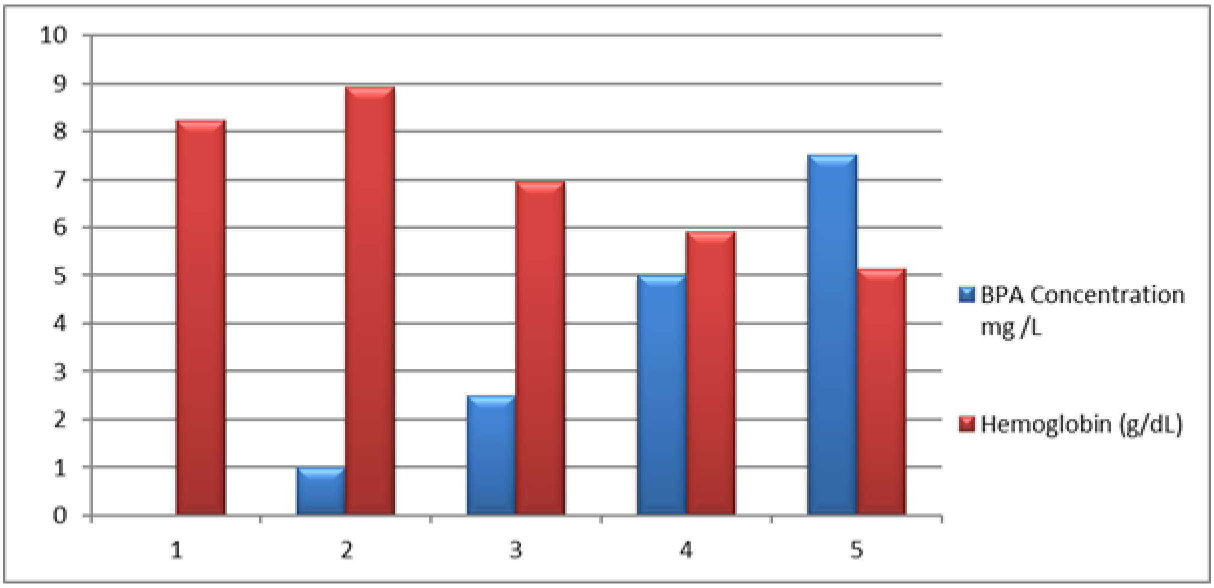

Figure 3. Present figure showing the bar chart between the BPA (mg/L) and Hemoglobin (g/dL) of *Oreochromis mossambicus* during 20 days of exposure to different concentrations of BPA.

Figure 3 showed the BPA exposure affects the Hb concentration of *O. mossambicus*, a fish that can indicate oxygen-binding and metabolic activity. The results show that higher BPA levels lead to lower Hb levels in the fish. The lowest Hb level (5.14 g/dL) was found in the group exposed to 7.5 mg/L of BPA, while the highest Hb level (8.93 g/dL) was found in the group exposed to 1 mg/L of BPA. The control group had a Hb level of 8.24 g/dL. The results imply that BPA harms the blood and physiology of *O. mossambicus*, and lowers their oxygen use and energy output. The results agree with previous studies on BPA and fish blood parameters

Table 2 Effects of Bisphenol A on Hematological Parameters of *Oreochromis mossambicus* after 20 Days of Exposure.

S. No.	Concentration of BPA	Pack cell volume (%)	MCHC	MCH	MCV
1	Control	25.02	32.93	47.36	143.79
2	Solvent control	26.18	33.69	53.78	159.63
3	1.0 mg /L	24.52	32.34	50.51	156.18
4	2.5 mg /L	22.68	30.60	45.06	147.27
5	5.0mg /L	19.57	30.20	41.33	136.85
6	7.5 mg /L	19.03	27.01	41.79	154.72

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The table 2 showed the effects of bisphenol A (BPA) on hematological parameters of *Oreochromis mossambicus* after 20 days of exposure. The results indicate that BPA has a negative impact on the blood parameters of the fish, as the hemoglobin (Hb), packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) values decreased significantly with increasing concentrations of BPA. These findings are consistent with previous studies that reported similar effects of BPA on hematological parameters of other fish species, such as Cyprinus carpio, Labeo rohita, and Carassius auratus. The decrease in Hb and PCV values indicates that BPA causes anemia in the fish, which may impair their oxygen-carrying capacity and affect their metabolism and growth.

The decrease in MCHC, MCH, and MCV values suggests that BPA affects the size and shape of the red blood cells, which may also impair their function and survival. The possible mechanisms of BPA-induced hematological alterations may involve oxidative stress, endocrine disruption, and immune suppression. Therefore, BPA poses a serious threat to the health and well-being of Oreochromis mossambicus and other aquatic organisms.

4. Conclusion

We reported that the RBC and Hb values of BPA-exposed fish were lower than those of control fish, implying reduced oxygen transport and anemia. The WBC and DLC values of BPA-exposed fish did not vary much from those of control fish. The effects of BPA exposure on the blood parameters were dependent on the dose and duration of exposure, with higher doses and longer exposures causing more damage. Our findings suggest that BPA exposure can impair the survival and health of fish by inducing oxidative stress and immune dysfunction.

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