International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

# Effects of Bisphenol A (BPA) on Haematological Parameters in the African Catfish, *Clarias Gariepinus*

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**Abstract:** ***Bisphenol A (BPA), a widely used chemical in polycarbonate plastics and epoxy resins, can enter aquatic environments through plastic waste and leaching from landfills. This study evaluated the effects of BPA exposure on selected haematological parameters in African catfish (Clarias gariepinus). Fish were acclimatised for one week and then exposed to BPA concentrations of 5, 10, and 15 mg/L for 15 days. Blood samples were analysed for total erythrocyte count, total leucocyte count, haemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration. BPA exposure caused marked, concentration-dependent alterations in blood parameters. Total erythrocyte count, haemoglobin concentration, packed cell volume, and mean corpuscular volume decreased significantly in fish exposed to higher BPA concentrations. In contrast, total leucocyte count, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration increased, with significant changes observed at 10 and 15 mg/L. The reduction in erythrocyte count, haemoglobin, and packed cell volume indicates impaired erythropoiesis and an anaemic condition, while the decreased mean corpuscular volume suggests microcytic anaemia. Increased leucocyte count indicates an immune response to BPA-induced stress. Overall, the findings demonstrate that BPA exposure adversely affects the haematological profile of C. gariepinus and that these parameters can serve as useful biomarkers for assessing BPA toxicity in aquatic organisms.***

**Keywords:** Bisphenol A, African catfish, Haematological parameters, Aquatic toxicity, Blood biomarkers, *C. gariepinus*

## 1. Introduction

Water bodies, including oceans, rivers, lakes, and ponds, support a rich diversity of living organisms and form aquatic ecosystems. The plants, animals, and microorganisms in these ecosystems rely on both living and non-living factors to survive. Aquatic ecosystems also play essential roles in maintaining environmental balance by recycling nutrients and naturally cleaning water. In addition, they provide opportunities for recreation and are an important part of the tourism industry.

An aquatic ecosystem becomes unhealthy when environmental stress exceeds its capacity to recover. Such stress may result from physical, biological, or chemical changes in the environment (Carpenter et al., 2011). In recent decades, human activities have greatly increased aquatic pollution due to the rapid growth of industrial, commercial, and domestic waste. Industrial development has made environmental pollution a major concern in India and across the world (Paul et al., 2012). Domestic sources also release large quantities of pollutants into water bodies, including soaps, detergents, vegetable waste, leftover food, and plastics. Plastics are particularly harmful because they may contain hazardous chemicals, such as phthalate derivatives and bisphenol A.

Bisphenol A (BPA) is a chemical mainly used to manufacture polycarbonate plastics and epoxy resins. It is also used in the production of polyester, polysulfone, and polyacrylate resins, as well as certain flame retardants. Polycarbonate plastics are commonly found in food-contact products such as infant feeding bottles, tableware, microwave-safe containers, food storage containers, water bottles, beverage bottles, processing equipment, and water pipes. Epoxy resins are often used as protective linings in food and beverage cans and as coatings on metal lids for glass jars and bottles, including containers used for infant formula (INFOSAN, 2009).

As plastic use continues to increase, greater amounts of bisphenol A (BPA) are released into the environment. BPA leaching is a serious concern because the chemical is present not only in plastic waste but also in water bodies, where it is regarded as a major contaminant. In addition, BPA can seep from soil into water sources due to plastic waste deposited in landfills.

Blood is a reliable indicator of an organism's health, and haematological parameters are valuable for assessing the functional condition of animals exposed to toxic substances (Mekkawy et al., 2011). Because the circulatory system interacts closely with the external environment, changes in blood variables can help reveal the effects of toxicants. Examining these parameters therefore provides useful information about the overall health of exposed organisms. Several studies have reported chemical-induced changes in the haematological parameters of fish (Aiswarya and James, 2016; Adedji et al., 2009), suggesting that such changes can be used to detect pollutant toxicity. In this context, the present study investigated the effects of different concentrations of bisphenol A on selected haematological parameters in the African catfish, *Clarias gariepinus*.

## 2. Materials and Methods

Fish used in the study were obtained from a local fish market. They weighed 150–300 g and measured 30–40 cm in length. The fish were maintained in glass aquaria containing 30 L of tap water under normal light and temperature conditions. After a one-week acclimatisation period, they were assigned to control and experimental groups exposed to BPA concentrations of 5, 10, and 15 mg/L.

After 15 days of exposure, the fish were collected using a hand net and immediately anaesthetised with 2-phenoxyethanol. Blood samples were obtained by severing the caudal vein and collected in EDTA-containing tubes. The samples were stored at 4 °C until analysis. Blood collection was performed according to the method described by Blaxhall (1972).

The quantitative changes in Total Erythrocyte Count (TEC), Total Leucocyte Count (TLC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Haemoglobin (MCH) were recorded. The estimation of total erythrocytes and leucocytes was done with the help of Neubauer chamber (Haemocytometer). The estimation of haemoglobin content was done by Sahli's Hemoglobinometer. The packed cell volume was estimated with the help of Wintrobe Hematocrit tube. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using the formulae mentioned by Dacie and Lewis (1991).

$$\mathrm{MCV\ (fL)} = \frac{\text{Packed Cell Volume (PCV)}}{\text{RBCs}}$$

$$\mathrm{MCHC\ (g/dl)} = \frac{\text{Haemoglobin (Hb)}}{\text{PCV}} \times 100$$

$$\mathrm{MCH\ (pg)} = \frac{\text{Haemoglobin (Hb)}}{\text{RBCs}} \times 10$$

## 3. Results and Discussions

Haematological parameters in fish are suitable biomarkers for evaluating the potential risk of pollutants and also to assess the status of fish health under stressful conditions. Blood cell indices are good indicators of systemic response to external stress and any changes is reflected in their distribution in the blood (Mekkawy *et al*. 2011).

**Total Erythrocyte Count (TEC)**

In the present study significantly lower values of erythrocyte count were observed when compared to control group. The RBC values decreased from 245±11.1 in the control group to 151±14.2 in 5mg/L BPA, 101±21 in 10mg/L BPA and 76±13 in 15mg/L BPA groups after exposure for 15 days (Table I and Fig. 1).

**Total Leukocyte Count (TLC)**

In this study there was a significant increase ($p<0.05$) in leukocyte count in all the test groups (40.4±2.16 in 5mg/L BPA; 61.5±2.93 in 10mg/L BPA; 89.1±3.21 in 15mg/L BPA) following exposure to different concentrations of BPA compared to control group (21.2±5.1) (Table I and Fig. 2).

**Haemoglobin (Hb)**

In the present study a significant decrease in the Hb concentration was observed in all the groups exposed to varying concentrations of BPA: 7.8±1.72 (5mg/L), 6.9±1.5 (10mg/L) and 5.6±1.74 (15mg/L) when compared to the control group (9.8±1.4) as shown in Table I and Fig. 3.

**Packed Cell Volume (PCV)**

In the present study a significant reduction in the PCV ($p<0.05$) was observed in 5mg/L (16.31±4.1), 10 mg/L (10.33±3.41) and 15 mg/L (7.3±1.9) of BPA treated groups, when compared to the control group (27.1±3.12) (Table I and Fig. 4).

**Mean Corpuscular Volume (MCV)**

In this study MCV decreased in all the treated groups 108.01±1.9, 102.27±1.6 and 96.05±1.71 in 5mg/L, 10mg/L and 15mg/L respectively when compared to the untreated group (110.61±1.52). The decrease in the MCV was insignificant in the 5mg/L BPA group. In the 10mg/L and 15mg/L BPA group the decrease observed was significant ($p<0.05$) (Table I and Fig. 5).

**Mean Corpuscular Haemoglobin Concentration (MCHC)**

In the present study MCHC showed a non-significant increase in the 5 mg/L BPA group (47.82±1.6), whereas significant increase ($p<0.05$) in the MCHC in the 10 mg/L (66.79±1.2) and 15 mg/L (76.71±1.4) BPA treated groups were found compared to the control (36.16±1.4) (Table I and Fig. 6).

**Mean Corpuscular Haemoglobin (MCH)**

In this study the MCH values increased insignificantly in the 5 mg/L BPA group (51.65±1.8) and significantly ($p<0.05$) in 10 mg/L (68.31±1.3) and 15 mg/L (73.68±1.11) BPA groups when compared with the control group (40±1.1) (Table I and Fig. 7)

In the present investigation, the fishes exposed to the sublethal concentration of BPA showed considerable alteration in the level of different blood parameters. These results were supported by the work of Kankaya and Kaptaner (2016). They observed a decrease in the RBC count and Hb content and increase in the WBC content in rainbow trout injected with nonylphenol similar to the present study. But on the contrary an increased PCV, MCV and decreased MCHC and MCH were observed.

Yaghoobi *et al.* (2017) also observed reduction of RBC, Hb and PCV in *A. latus* exposed to BPA. The reduction of RBC in exposed fish to BPA could be related to the increase of free radicals in fish blood via inhibition of the antioxidant enzymes by this compound. This process leads to lipid peroxidation in blood cells which in turn may produce oxidative stress in the body and affect the cell membrane of red blood cells, finally leading to the collapse of these cells. Fish exposed to 5, 10, and 50 µg/g of BPA showed a significant decrease ($P < 0.05$) of the MVC and MCH in 14 days, while this reduction in 21 days was observed in all treatments compared to the control group. MCHC revealed a significant decrease ($P < 0.05$) in 50 µg/g BPA only for 14 days, and in 21 days, the same result was observed in 5, 10, and 50 µg/ g BPA compared to the control group.

George *et al.* (2017) also observed variations in the blood parameters of *Channa striatus* exposed to sublethal concentrations of diethyl phthalates (DEP). Decrease in RBC count, WBC count, Hb, PCV, MCV and MCH were observed. The reduction in the RBC count of DEP exposed fish may be most likely due to the physiological effect of haemopoietic system, which is a susceptible marker related to environmental contaminants. The decrease in the Hb content is a sign of weakened oxygen transport which may occur due to destruction of the RBCs. The change in the WBC count is a result of the immune toxicity due to DEP.

The results in the present study are in agreement with the haematological changes such as anaemia marked by significantly low value ($p<0.05$) of RBC, Hb, PCV was reported in findings of Adeyemo (2005) in *C. gariepinus* due to effects of Cassava mill effluent. Significantly low MCV, MCH and MCHC values were observed in the exposed group compared to the baseline group. The decreased MCV with reduced Hb content signifies the shrinkage of the red blood cells (microcytic anaemia). Microcytosis may be due to decreased PCV during exposure. Fluctuation in MCH and MCHC indicates fluctuation in the concentration of haemoglobin in the exposed groups.

As observed in the present study reduction in the RBC count, PCV and Hb content signifies erythropenia (suppression of erythopoiesis) and anaemic condition in fish. MCV, MCH, and MCHC values are useful in elucidating the etiology of anaemia. The MCHC is a useful indicator of RBC swelling and/or to a decrease in haemoglobin synthesis (Yaghoobi *et al.*, 2017).

In the present study the decrease in the RBC count, PCV and Hb content signifies that the RBC production is hampered due to effect of BPA. Also decreased MCV signifies microcytic anemia i.e. shrinkage of RBC. However increased value of MCH and MCHC signifies increased concentration of haemoglobin (hyperchromia) in the red blood cell. An immune response to the toxic chemical is signified by the increase in the WBC count in the exposed groups.

**Table I:** Haematological parameters of *Clarias gariepinus* exposed to sub-lethal concentrations of Bisphenol A for 15 days.

| | Control | 5mg/L | 10mg/L | 15mg/L |
|---|---|---|---|---|
| TEC×$10^4$/mm$^3$ | 245 ± 11.1 | 151±14.2* | 101±21** | 76±13*** |
| TLC× $10^3$/mm$^3$ | 21.2 ± 5.1 | 40.4 ± 2.16* | 61.5±2.93** | 89.1±3.21*** |
| Hb (g/dl) | 9.8±1.4 | 7.8 ±1.72NS | 6.9 ±1.5* | 5.6±1.74* |
| PCV (%) | 27.1±3.121 | 16.31±4.1* | 10.33±3.41** | 7.3±1.9*** |
| MCV (fL) | 110.61±1.52 | 108.01±1.9NS | 102.27±1.6* | 96.05±1.71** |
| MCHC (g/dl) | 36.16±1.4 | 47.82±1.6NS | 66.79±1.2* | 76.71±1.4** |
| MCH (pg) | 40±1.1 | 51.65±1.8NS | 68.31±1.3* | 73.68±1.11** |

Values are presented as Mean ± S.D; **N=** 05 for each group, $p<0.05$*, $p<0.001$**, $p<0.0001$***, NS= Not significant
TEC – Total Erythrocyte Count; TLC – Total Leukocyte Count; Hb – Haemoglobin; PCV – Packed Cell Volume; MCV – Mean Corpuscular Volume; MCHC – Mean Corpuscular Haemoglobin Concentration; MCH – Mean Corpuscular Haemoglobin

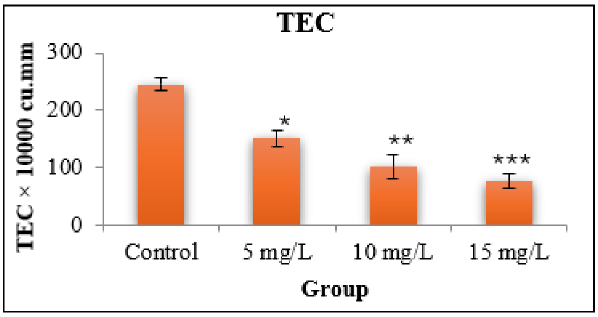


**Figure 1:** Effect of BPA on Total Erythrocyte Count (TEC) of *Clarias gariepinus* exposed to sub-lethal concentrations of Bisphenol A for 15 days. (Mean±S.D) $p<0.05$*, $p<0.001$**, $p<0.0001$***

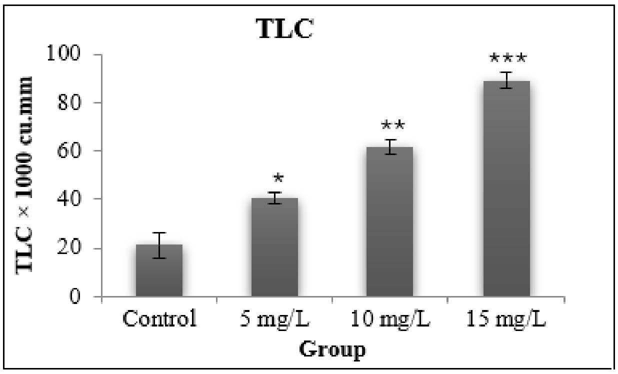


**Figure 2:** Effect of BPA on Total Leucocyte Count (TLC) of *Clarias gariepinus* exposed to sub-lethal concentrations of Bisphenol A for 15 days. (Mean±S.D) $p<0.05$*, $p<0.001$**, $p<0.0001$***

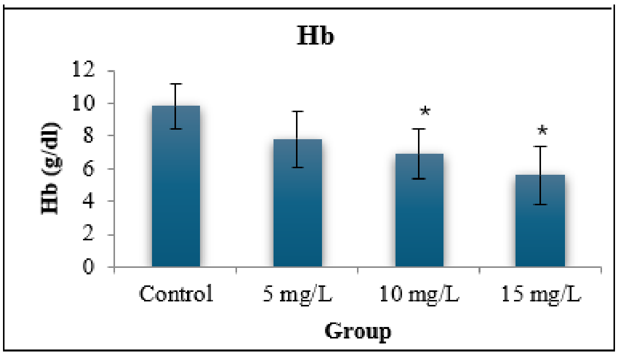


**Figure 3:** Effect of BPA on Haemoglobin concentration (Hb) of *Clarias gariepinus* exposed to sub-lethal concentrations of Bisphenol A for 15 days. (Mean±S.D) $p<0.05$*, NS= Not significant

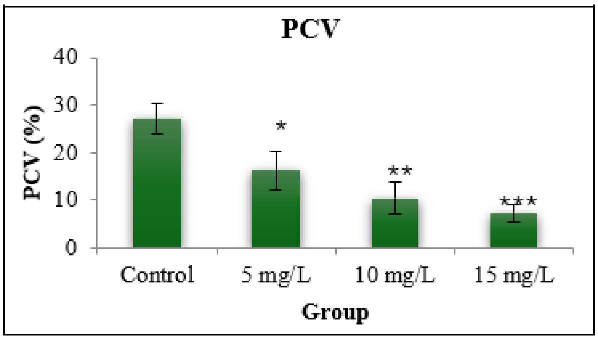


**Figure 4:** Effect of BPA on Packed Cell Volume (PCV) of *Clarias gariepinus* exposed to sub-lethal concentrations of Bisphenol A for 15 days. (Mean±S.D) p<0.05*, p<0.001**, p<0.0001***

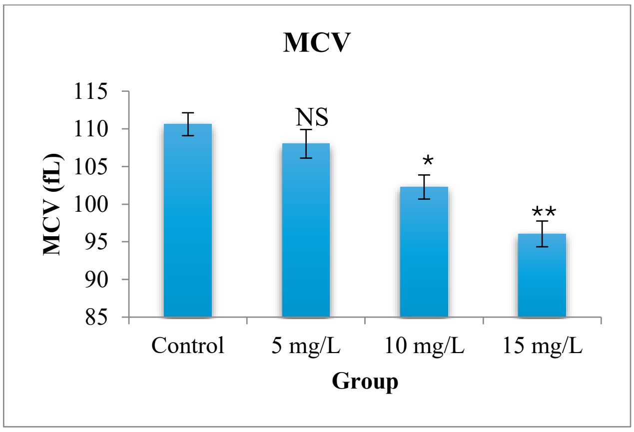


**Figure 5:** Effect of BPA on Mean Corpuscular Volume (MCV) of *Clarias gariepinus* exposed to sub-lethal concentrations of Bisphenol A for 15 days. (Mean±S.D) p<0.05*, p<0.001**

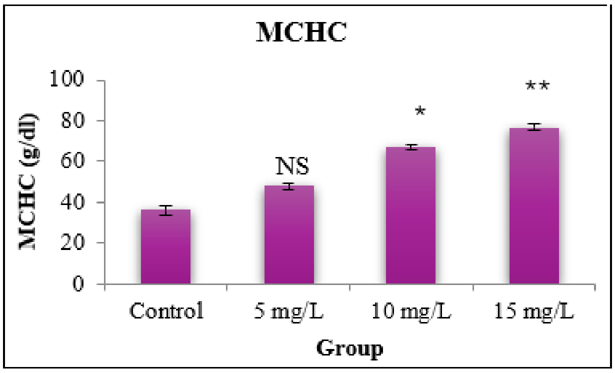


**Figure 6:** Effect of BPA on Mean Corpuscular Haemoglobin Concentration (MCHC) of *Clarias gariepinus* exposed to sub-lethal concentrations of Bisphenol A for 15 days. (Mean±S.D) p<0.05*, p<0.001**

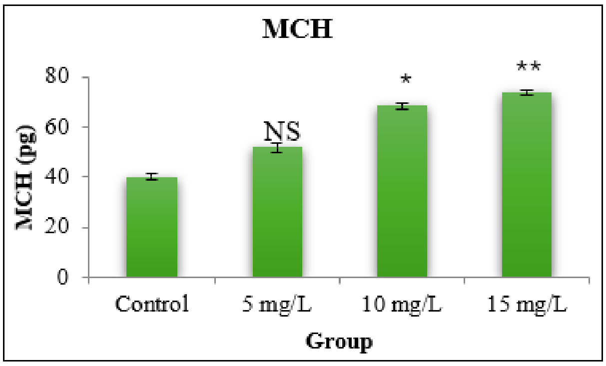


**Figure 7:** Effect of BPA on Mean Corpuscular Haemoglobin (MCH) of *Clarias gariepinus* exposed to sub-lethal concentrations of Bisphenol A for 15 days. (Mean±S.D) p<0.05*, p<0.001**

## 4. Conclusion

Haematological parameters in fish are suitable biomarkers for evaluating the potential risk of pollutants and also to assess the status of fish health under stressful conditions. In the present study a significant decrease was observed in the total erythrocyte count, haemoglobin concentration, packed cell volume and mean corpuscular volume. The total leucocyte count, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin showed a significant increase. The decrease in the total erythrocyte count, haemoglobin concentration and packed cell volume signifies that the RBC production is hampered due to effect of BPA. Also decreased mean corpuscular volume signifies microcytic anemia i.e. shrinkage of RBC. However increased value of mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin signifies increased concentration of haemoglobin (hyperchromia) in the red blood cell. An immune response to the toxic chemical is signified by the increase in the total leucocyte count in the exposed groups.

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