



ECO-TOXICOLOGICAL INSIGHTS: MODULATING BISPHENOL A-INDUCED HEMATOLOGICAL CHANGES WITH FLAXSEED DIETS IN FOOD FISH *LABEO ROHITA*

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ABSTRACT

The present study was aimed to assess the effects of dietary flaxseed supplementation in mitigating the toxic impacts of Bisphenol A on the haematological parameters of food fish *Labeo rohita* (rohu) (Hamilton, 1822). Bisphenol A, a chemical integral to plastic manufacturing, has potential to impact humans and aquatic fauna. The experiment involves three groups: A, B, & C. The group A was served as control group, while group B was exposed to BPA dose and fed a standard diet. Group C received the same BPA dose as Group B but supplemented with a flaxseed- enriched diet. Blood samples were collected at 7th, 14th, and 21st days. Haematological parameters, including red blood cells count (RBC), haematocrit (hct) & Haemoglobin concentration (Hb), platelets count, white blood cells (WBC) count and blood indices like mean corpuscular haemoglobin (MCH) & mean corpuscular volume (MCV) were analysed. The BPA treated group shows significant ($p < 0.05$) changes, reduction in values of RBC, Hb, Hct, MCV and Platelets, while the value of WBC and MCH get increased. Group C shows significantly ($p < 0.05$) increase in Hb, RBC & platelet count, Hct percentage and in MCV value from group B. The WBC count also get increase in group C as compare to control group. The study highlights that a flaxseed diet positively impacts hematological parameters in *L. rohita* exposed to BPA toxicity. Incorporating bioactive compounds like flaxseed into fish diets enhances their health and resilience against waterborne toxicants. Additionally, hematological parameters are identified as reliable biomarker for assessing fish health in ecotoxicological research.

Keywords: Bisphenol A, Ecotoxicology, Flaxseed, Haematology Labeo, Stress.

INTRODUCTION

The legacy of plastic has led to a flood of plastic waste. The plastic constituents are gradually released into the environment due to overuse of plastic products. Extensive analytical studies conducted globally have generated a comprehensive dataset on the presence of plastic particles across various environments and matrices. Bisphenol A (BPA) is a synthetic chemical, integral in making toughen plastic sheet, other plastic products, and epoxy resins. BPA is found in a range of everyday products, including food containers, water bottles, and even in the linings in canned goods. BPA is one of the most common endocrine disruptors. Mimicking the hormone estrogen, BPA can interfere with the normal functioning of the endocrine system, leading to the potential disturbances in

reproductive health, metabolic processes, and developmental outcomes. BPA is released from plastic into the aquatic environment in several ways. It releases into aquatic environment from house hold sewage, & direct from manufacturing factories. The biggest reason for spreading of BPA to every pond, river and in oceans also, is microplastic (Koelmans *et al.*, 2019; Schymanski *et al.*, 2021) and plastic litters. From these BPA directly or indirectly reaches into aquatic fauna. Fish is highly responsive to pollutants and toxins present in water and are widely recognized as reliable and practical model for assessing the toxic effects of environmental pollutants. BPA is found in abundance in fish body because of its entry through gills, oral ingestion and through diet.

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Moreover, according to one research, exposures to plastic particles in humans can lead to their entry and absorption into the blood stream (H. A. Leslie *et al.*, 2022). This also reflects that plastic particles reaches into the blood stream of fish & direct release BPA into blood. So, the percentage of BPA in fish body and blood remains high. This BPA exposure would lead to notable alterations in hematological parameters of fish (Meghwal & Gupta, 2023; Aishwarya & James, 2016). Hematological parameters serve as indicators of blood physiology and the body stress levels, reacting sensitively to toxic substances. In our present study we have used two compounds, BPA, and flaxseed respectively against each other, to evaluate the hematological parameters of fresh water food fish *Labeo rohita* (rohu). Very few studies have investigated the hematological responses of fish against BPA and flaxseed exposure independently. *L. rohita* (rohu) (Hamilton, 1822) was chosen as experimental model for present study because it is extensively cultivated as food fish and have great economic importance. Rohu is found in almost all riverine systems of India and also popular in pond culture under polyculture (Majumder *et al.*, 2018)

Different studies and experiments have been done so far to check on fish health and growth status by using different beneficial organic compounds, herbs, and oil supplements. As we have not found any data regarding these two compounds i.e., BPA and flaxseed combination in fish ecotoxicology. We hypothesized that, by including flaxseed, in the diet of fish, which is a bioactive compound and a rich source of Omega 3 can possibly reduce or prevent the toxic effects of BPA. So, the present investigation was undertaken to understand the beneficial effects of flaxseed diet on blood parameters of *L. rohita* against BPA toxicity.

MATERIALS AND METHOD

Rohu fish were sourced from local ponds and housed in separate aquarium tanks, with each tank containing 12 fish for control and treatment groups. The specimens were acclimated to laboratory conditions for period of 7 days prior to the commencement of the experiment. The experiment runs for 21 days. Three groups were established A, B, and C. The group A is treated as control group, group B is treated with BPA dose and readymade feed and group C is treated with same amount of BPA dose as in group B along with flaxseed diet. The tanks were filled with water up to 40 l, and aeration was maintained throughout the experiment. The dose of BPA was decided to give 8ppm which is 1/4th of LC 50. Bisphenol A was obtained from LABOGENS Fine Chem industry, Punjab. Since BPA has

low solubility in water, solvents like dimethyl sulfoxide or ethanol are typically used to dissolve it. In this study, ethanol was chosen as solvent for dissolving BPA. High quality flaxseed was sourced from the local market and processed into a fish diet by grinding it and blending it with rice bran, wheat bran, and other essential ingredients. The final formulation included 30g of flaxseed per 100g of feed.

Blood collection and hematological analyses

The blood was collected at 7th day, 14th day and 21st days of the experiment from each of the three groups. Blood samples were drawn from the caudal vein and collects in EDTA tubes. The blood samples were used for the estimation of total erythrocyte count (TEC), Haemoglobin concentration (Hb), haematocrit (HCT), white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and platelets. The values of samples were measured by an automatic haematology Sysmex analyser.

Statistical analysis

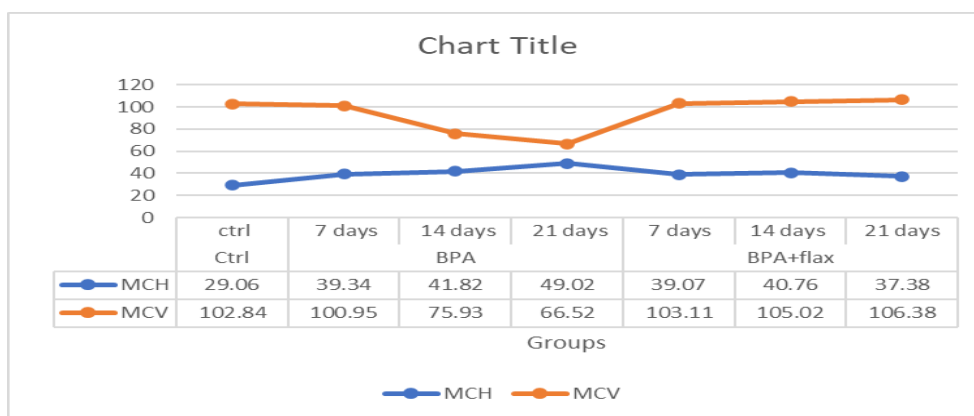
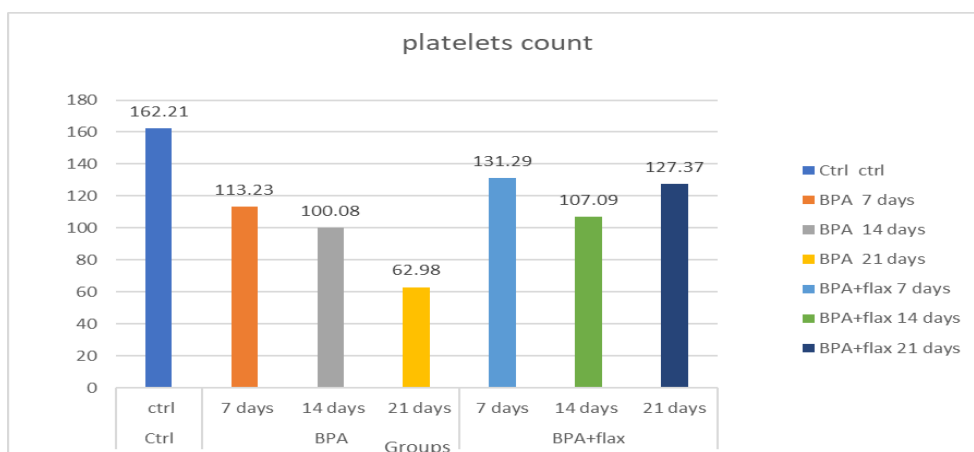
All data were expressed as the mean along with the standard deviation. All data set are in uniform distribution thus, Kruskal-Wallis test was employed to determine the significance level ($p < 0.05$) of the hematological assay variables. Serving as a non-parametric alternative to ANOVA, the Kruskal-Wallis test evaluates mean difference across multiple independent groups, with the analysis conducted using IBM SPSS statistics (version 25).

RESULTS AND DISCUSSION

The values of Hb, RBC, Hct, and platelets get reduced significantly ($p < 0.05$) in both groups B and C at 21st day from the control group. The increase in Hb% is seen in group C than the group B at all 3 marked days. The RBC count increased significantly in group C (3.82 ± 0.03) at 21st day than in group B (2.75 ± 0.01). Similarly, Hct% and platelets counts were found significantly ($p < 0.05$) higher in group C at 21st day in comparison to group B. In contrast the values of WBC and MCH get increased in both the treated group as compared to control group A. There is no significant difference in WBC count in Group B and group C at 21st day. The results are shown in Table 1. The value of MCH in group C (42.47 ± 0.1) is significantly ($p < 0.05$) low from group B (49.82 ± 0.2) on 21st day. The value of MCV get significantly reduced in group B as compared to control group A, but MCV level get significantly increase in group C from group B as well as from control group (Figure1). The platelets count was found highest (162.21 ± 2.1) in control group. Group B has showed lowest (62.98 ± 2.1) platelet count at 21st day, while group C shows significantly ($p < 0.05$) (127.37 ± 1.6) higher value than group B at 21st day (Figure 2).

Table 1. Effect of BPA (group B) alone and BPA + flaxseed (group C) diet on some haematological parameters of fresh water fish *L. rohita*. The values are represented in (mean $\pm$ SE).

Parameters /	Groups		
Exposure period in days	Control (A)	BPA (B)	BPA+ Flax (C)
Haemoglobin(g/dl)			
7	9.83 $\pm$ 0.00	9.57 $\pm$ 0.03	9.62 $\pm$ 0.04
14	9.79 $\pm$ 0.00	9.17 $\pm$ 0.02	9.41 $\pm$ 0.05
21	9.87 $\pm$ 0.00	8.57 $\pm$ 0.1	9.26 $\pm$ 0.1
Red blood cells (x10 ⁶ /mm ³)			
7	4.40 $\pm$ 0.01	3.56 $\pm$ 0.2	4.13 $\pm$ 0.07
14	4.46 $\pm$ 0.01	3.47 $\pm$ 0.2	4.09 $\pm$ 0.2
21	4.5 $\pm$ 0.01	2.75 $\pm$ 0.01	3.82 $\pm$ 0.03
White blood cells (x10 ³ /mm ³)			
7	8.96 $\pm$ 0.2	13.42 $\pm$ 0.02	13.70 $\pm$ 0.04
14	8.90 $\pm$ 0.2	14.44 $\pm$ 0.2	12.32 $\pm$ 0.01
21	8.93 $\pm$ 0.2	15.40 $\pm$ 0.3	16.41 $\pm$ 0.1
Haemocrit (%)			
7	42.89 $\pm$ 0.5	39.03 $\pm$ 0.2	39.91 $\pm$ 0.08
14	40.33 $\pm$ 0.4	37.14 $\pm$ 0.3	36.23 $\pm$ 0.1
21	41.78 $\pm$ 0.6	30.51 $\pm$ 0.2	34.18 $\pm$ 0.3

**Figure 1.** Effect of BPA and BAP + flaxseed diet on two blood parameters MCH (pg) (blue), and MCV (fl) (orange). The dots show respective values at respective days in groups. (data table is also given).**Figure 2.** Effect of BPA and BPA + Flaxseed diet on the platelets count (10⁹/L) in three groups at 7th, 14th, & 21st days.

Aquatic toxicity tests are commonly employed to evaluate the toxic effects of chemicals or pollutants on aquatic organisms and to ensure their safe and limited use with their proper disposal (Krishnapriya *et al.*, 2017). The current study provides insights into the impact of Bisphenol A and flaxseed supplementation on the hematological parameters of fish, emphasizing the interplay between toxic exposure and nutritional intervention. The health of fish can be assessed through hematological parameters, which highlight both their physiological state and their reactions to environmental stressors (Fazio F., 2019; Acharya & Mohanty, 2014). Exposure to BPA led to a reduction in haemoglobin content and red blood cell count along with an increase in WBC count in fish (Sharma & Chadha, 2012; Aiswarya & James, 2016; Minaz *et al.*, 2022). The present study shows similar results related to above studies. A decline in Hb% in current study could be result from its breakdown or diminished synthesis (Mostakim *et al.*, 2015). Variation in haemoglobin concentration in experimental fish may result from injury, cellular damage, or disruptions to the hematopoietic tissues (Kumar & Banerjee, 2016). A notable reduction in Hb content was detected in rainbow trout subjected to diazinon exposure during (Far *et al.*, 2012) the experiment and in *Channa punctatus* due to contact with carbofuran (Ashaduzzaman *et al.*, 2016).

The reduction in RBC count in current study exposed to BPA may be attributed to several factors. BPA exposure can induce oxidative stress, damaging erythrocytes and shortening their lifespan or erythrocyte destruction due to increased production of reactive oxygen species (ROS) (Wu & Seebacher, 2020). Moreover, BPA induced damage to the organs such as kidney and liver, which are involved in waste management and erythropoiesis, can impair RBC production and survival (Smorodinskaya S. *et al.*, 2023; Faheem *et al.*, 2019; Pal & Reddy, 2018). The findings of the present study align with the result reported by Keum *et al.* (2005) in Korean rockfish, *Sebastes schlegeli*. Abnormally low or high Hct levels could indicate the presence of a blood disorder. A marked reduction in Hct value in group B suggests, anaemia in fish under stress caused by BPA toxicity. When *Labeo* was exposed to azo dye Bismarck brown (Gupta P., 2013) and *Heteropneustes fossilis* on exposed to BPA (Srivastava & Reddy, 2020) significant decrease was seen in Hct%. In group C the results of Hb, RBCs, and of Hct get significantly high from the group B, demonstrated that incorporating a flaxseed diet led to the improvements in haematological parameters of the fish. The results are also pointing that, the flaxseed components are possibly capable of reduce the harmful effects of BPA or can fight against the toxicity caused due to BPA. A significant increase in RBC, WBC, Hb, and platelets was showed up when *L. rohita* fingerlings were supplemented with flaxseed-based probiotics (Shahzad *et al.*, 2021). Rajikkannnu *et al.*, (2015) reported significant increase in RBCs count, Hb, Hct percentage, and RBC indices like MCV and MCH when fish fed with supplemented diet. The values of Hb, RBC, and Hct of group C shows significant reduction from control group because of BPA negative effects.

WBC counts increase in both groups B and C compare to control group. WBC counts in fish increase under various conditions, primarily as a physiological response to stress, infection, or environmental challenges. In present study increase in WBC count in fish in group B (exposed to BPA), is primarily a defence response to the chemical's toxic effects. This indicates BPA induces toxic stress and cellular damage, which trigger the generalised immune system of fish. Consistent with the findings of this study, a notable rise in white blood cell count was observed in *Labeo* (Ghaffar *et al.*, 2020) when exposed to Thiamethoxam and in *Channa punctatus* after BPA exposure (Sharma & Chadha, 2021), while WBC level was raised in *Oreochromis mossambicus* after experience BPA doses (Meghwal & Gupta, 2023). A study done by Prasad and Stoglin (2005) reported that flaxseed diet when given to healthy humans does not alter WBC count. On the contrary the addition of flaxseed powder and oil in the diet of streptozotocin induced diabetic rat, normalized the WBC count, indicating flaxseed's potential to restore immune balance (Harby, 2023). Banerjee S. *et al.*, (2023) shows that level of WBC get increase when the linseed oil cake is used in fish diet along with fermented fish gut bacterium (*Bacillus pumilus*). Group C results are in accordance with the findings of Page & Deshai, (2016).

Platelets count represents the number of platelets present in a specific volume of blood and is typically reported as platelets per cubic millilitre of whole blood. Platelets are crucial to the intrinsic coagulation system due to adhesive properties and capacity to aggregate. Following vascular injury, they attach to the subendothelial matrix of the blood vessel wall and form clusters with other thrombocytes (Ortiz & Esteban, 2024). The current study shows decline in platelets count in both the treated groups (B & C). In group B the platelet count reduced too much from control group. The toxic effects of BPA cause tissue damage or haemorrhage resulting a compensatory mechanism by platelets aimed at countering potential excessive bleeding, might be a possible reason for reduction of platelets count in fish (Sinha *et al.*, 2022). *H. fossilis* showed significant drop in platelet count when exposed to fenthion (Shrivastav & Mishra, 1983). A substantial decrease in platelet level was observed in *Channa punctatus* due to carbofuran (Ashaduzzaman *et al.*, 2016). In flaxseed+ BPA treated group the value of platelets is significantly increase from the group treated with BPA alone. When BPA and flaxseed diet are administered together to fish, the nutrients in flaxseed may support overall health and enhance the resilience of the hematopoietic system against BPA induced toxicity. Moreover, flaxseed lignans and alpha-linolenic acid, which have antioxidant properties, (Kausar S. *et al.*, 2024; Kajla *et al.*, 2015) possibly mitigate BPA induced hematological changes.

MCV and MCH are erythrocytes secondary indices which are essential for understanding RBC characteristics and used to identify different types of anaemia (Sinha *et al.*, 2022). MCV reflects the average volume of RBCs,

provides their size information. While MCH measures the average haemoglobin content per RBC. Studies investigating impact of various harmful, chemicals, pesticides, or endocrine disruptors on teleost haematological parameters. However, these studies reveal no definitive or consistent trends in the variation of absolute values of MCV and MCH (Sinha *et al.*, 2022). The present study shows significant decreasing values of MCV in group B as compared to group A. It represents the condition of microcytosis, in which red blood cells size get smaller. Iron deficiency and anaemia is the most possible cause of low MCV values (Massey, 1992). Our findings were correlated to Krishnapriya *et al.*, (2017) study where the MCV value was also found to be very low in *Labeo* when treated with BPA. Similarly, Yaghoobi *et al.*, (2017) observed low values of erythrocyte secondary indices in yellowfin seabream after prolonged exposure to BPA. While the results of Srivastav and Reddy, (2020) study, shows increase in MCV level in *H. fossilis* when exposed to BPA. However, no notable variation was observed for MCV in *O. mykiss* when prompted to BPA (Minaz *et al.*, 2022). The MCV level get significantly increase in group C as compared to control group and group B. This clearly shows that flaxseed improved nutritional status of fish. Flaxseed oil has demonstrated protective effects against BPA-induced toxicity in animal models, indicating its potential to counteract BPA's deleterious effects (Makawy A.E. *et al.*, 2018). Studies have shown that flaxseed supplementation can improve liver lipid metabolism and function markers (Li X. *et al.*, 2021, Yari Z. *et al.*, 2016), suggesting a supportive role in hepatic detoxification processes. Thus, flaxseed may promote detoxification pathways in the liver, reducing the bioavailability of BPA in the bloodstream and minimizing its impact on RBC. This may helpful in improving the MCV value in group C. The study done by Oluah *et al.*, (2020) present reduced MCV and MCH values in *Clarias* when get in touched in herbicide Ronster. In contrast to this in current study the MCH value was found highest in group B (49.02 ± 0.1). Group C has also higher value as compared to control group, but was significantly ($p < 0.05$) low (37.47 ± 0.1) from group B at 21st day. The MCH level remains unaffected while MCV values get reduced in *O. niloticus* when exposed to chlorpyrifos (Hossain *et al.*, 2022). BPA exposure could increase MCH values by causing stress on erythropoiesis (Meghwal & Gupta, 2023). In group C flaxseed diet may counteract the oxidative damage caused by BPA. It also provides essential fatty acids and micronutrients that might support the production of red blood cells with optimal haemoglobin content (Sembratowicz I. *et al.*, 2020).

CONCLUSION

The previously mentioned studies highlight that BPA significantly impacts the haematological parameters in fish

species, while the effects of a flaxseed diet on blood parameters of fish do not follow a consistent pattern. No direct studies currently show the collective effects of BPA and flaxseed on blood parameters of fish. Our study found that flaxseed nutritive diet is helpful in attenuating the negative impacts of BPA on haematological parameters of fish. Overall, the combined effect would depend on the relative concentrations of BPA and amount of flaxseed included in diet, as well as the duration of exposure. Moreover, incorporating nutritional strategies, such as flaxseed supplementation against toxicants is prove to be safeguard for aquatic health. Further experimental studies would be needed to establish consistent effects.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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