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# Hematological Alteration Induced in the Fish Grass Carp (*Ctenopharyngodon idella*) Exposed to Bisphenol A (BPA) Compound

Naqash Khan\*, Ahsan Khan, Aamir Sultan, Kareem Ullah, Asad Ali and Bushra Bibi

Department of Zoology, University of Swabi, Anbar, Swabi, Khyber Pakhtunkhwa, Pakistan

#### Abstract

Hematological indices of grass carp (*Ctenopharyngodon Idella*) were under the observation in the present experiment against the single concentration of Bisphenol A for 15 days. Observations were made from 3-15 days to study the differences in hematological parameter of control and treated groups. The blood parameters checked for 3, 6, 9, 12 and 15 days respectively against concentration of 0.30mlL-1 of bisphenol included white blood cells, haemoglobin, platelets and leucocytes count. White blood cell, haemoglobin and in differential leucocytes count lymphocytes, monocytes, neutrophils concentration against bisphenol A were increased as compared to the control group. . More increase was observed during 15 days exposure. Polymorphs concentration against toxicity of bisphenol A was decreased when compared with control group. Fish also showed behavioural changes like loss of balance, fast swimming and fast movement, equilibrium, vertical swimming etc. This parameter indicated to be used could be used as an index for evaluation of pathological condition of aquarium organisms.

Keywords: Hematology; Toxicological; RBC; WBC; TLC

# Introduction

Aquatic ecosystem running through agriculture or industrial area is likely to be contaminated by various chemicals leached from runoff and groundwater [1].

The presence of chemicals in the environment causes social and scientific development and their immense use can create risks to the environment (e.g aquatic bodies), resulting in damage to species specially fish [2].

The most far spread organism in aquatic bodies are fish and its susceptibility the climatic pollution may back the degree of biotic effect of environmental contamination of aquatic body. Observing the different parameter of blood at both cellular and acelluar, may be of considerable evaluation of the diagnostic value of early warning signals of pesticide poisoning [3].

Aquatic pollution has profound effects on organism the recipient environment. In the recants years due to the adverse effects of BPA on Wildlife, especially fish, BPA has been widely used in aquatic toxicity testing. Widely Fish are used to Determining the health status of aquatic organism because their biological responses are biomarkers of environmental pollution.

Bisphenol A (C15H1602) is an organic compound with two phenolic functional groups that is used along with other material used to make plastics to make polycarbonate polymers and epoxy resin. The final product of bisphenol A has been found in some domestic material, such as adhesives, protective coating, powder coating, automotive lenses, paper coating, thermal paper, etc. as a developing agents are used in the encapsulation of dye, electrical and electronic parts, and are also used as materials for the synthesis of polycarbonate and epoxy plastics. Widely used in food and beverage containers, dental material and paints [4].

The introduction of BPA impurities into the environment can cause serious environmental concerns as BPA can seriously affect human and animal health.

The widespread presence of BPA in various products leads to its continue release into aquatic ecosystem through leakage of polycarbonate plastics, posing Eco toxicological risks to the health of aquatic organisms [5].

Although it has short half-life in aquatic environment and effect on fish population, behavior, and central nervous system of fish and significantly impact growth, morphology, biochemical variables and histological structure of fish [6].

The aquatic ecosystem receives a vast spectrum of bisphenol A, which may be introduced directly or indirectly. A chemical is one of these pollutants which cause serious environmental problems [7].

When fish were exposed to lethal and sub lethal concentration of the two toxic agents, changes in blood parameters were observed as decreased in RBC, Hb and an increased in WBC [8].

Although much literature has considered the effects of anthropogenic pollutants on the behaviours of different fish species, hybridization and hyperactivity following BPA exposure are well studied in zebrafish [9].

#### Materials and Method

# Chemical, test animal and study duration

The Chemical of choice for the study id Bisphenol A (C15H1602) an organic compound with two phenolic functional groups used to make polycarbonate polymers and epoxy resins, among other material used to make plastic.

The proposed study was carried out at the Department of Zoology, University oof Swabi to assess the toxicological effect of BPA on grass

\*Corresponding author: Naqash Khan, Department of Zoology, University of Swabi, Anbar, Swabi, Khyber Pakhtunkhwa, Pakistan, E-mail: knaqash649@ gmail.com

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# Page 2 of 3

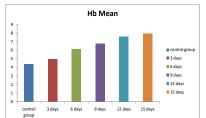


Figure 1a. Bar Diagram showing changes in the hemoglobin levels of grass carp (Ctenopharygdon idella) against the concentration of BPA treated group.

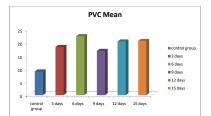


Figure 1c. Bar Diagram showing changes in the PVC levels of grass carp (Ctenopharygdon idella) against the concentration of BPA treated group.

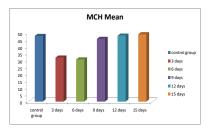


Figure le. Bar Diagram showing changes in the MCH levels of grass carp (Ctenopharygdon idella) against the concentration of BPA treated group.

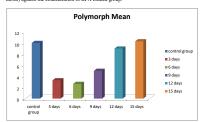


Figure 1g. Bar Diagram showing changes in the hemoglobin levels of grass carp (Ctenopharygdon idella) against the concentration of BPA treated group.

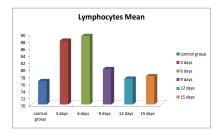


Figure 1i. Bar Diagram showing changes in the Lymphocytes levels of grass earp (*Ctenopharygdon idella*) against the concentration of BPA treated group.

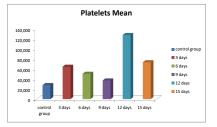


Figure 1k. Bar Diagram showing changes in the Platelets levels of grass carp (*Clenopharygdon* Figure 1L. Bar Diagram showing changes in the Monocytes levels of grass carp *idella*) against the concentration of BPA treated group. (*Clenopharygdon idella*) against the concentration of BPA treated group.

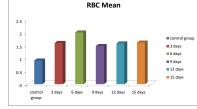


Figure 1b. Bar Diagram showing changes in the RBC levels of grass carp (Ctenopharygdon idella) against the concentration of BPA treated group.

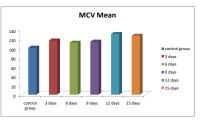


Figure 1d. Bar Diagram showing changes in the MCV levels of grass carp (*Ctenopharygdon idella*) against the concentration of BPA treated group.

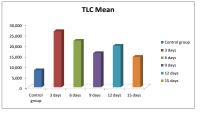


Figure 1f. Bar Diagram showing changes in the TLC levels of grass carp (Ctenopharygdon idella) against the concentration of BPA treated group.

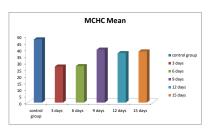


Figure 1h. Bar Diagram showing changes in the MCHC levels of grass carp (Ctenopharygdon idella) against the concentration of BPA treated group.

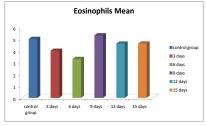
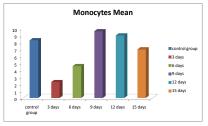


Figure 1j. Bar Diagram showing changes in the Eosinophils levels of grass earp (*Ctenopharygdon idella*) against the concentration of BPA treated group.



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carp (*Ctenopharyngodon Idella*) at single dose level of 0.30mL-1 at 3, 6, 9, 12 and 15 days.

# Test animal

Healthy and uniform size seeds of grass carp, normal body weight 3.23  $\pm$  0.34 g were bought and moved in oxygenated packs from Peshawar and Mardan carp hatchery and then were carefully analyzed and treated with 0.2% KMno<sub>4</sub> solution for 2 minutes to treat dermal infections. Fish were acclimatized for two weeks in aquarium having tap water and then were shifted to experimentation fiber glass tanks for the purpose of experimentation. Both in acclimatization and experimentation aquarium fish were properly fed with commercial food. Other water quality parameters were also checked on every alternated day during the experimentation and were found in permissible limits as per recommended values of APHA and American Public.

### Sampling of blood

After stipulated time three fish from every aquarium in the control and treated group were captured with a hand net and blood sample was collected by caudal severance from the fish during early hours of the day.

**Hematological parameters:** For examination of the hematological parameter, blood was drawn from the caudal vein of grass carp through sterile heparinized syringe and was stored in EDTA tubes. Blood samples were observed through hematological analyser Model No (URIT 3020) placed in laboratory of Department of Zoology, University of Swabi.

**Statistical analysis of collected data:** At the end of the research collected data was statistically analyzed. Statistical data was analyzed by calculating mean and standard deviation.

# **Results and Discussion**

The calculated  $(LC_{15})$  for 3, 6, 9, 12 and 15 days respectively with a confidence limit of bisphenol A to grass carp (*Ctenopharygdon Idella*) was 0.30ML<sup>2</sup> ppm indicating the moderate toxicity of BPA to the fish.

During above exposure period the fish shows various behavioural responses like increased opercula movement, mucous secretion, jerky movement, floating on the sides, hypersensitivity showing violent erratic and fast swimming etc.

The blood alterations of grass carp exposed to acute concentration of bisphenol A is shown in Figure 1. The concentration of RBC, HB, MCV, MCH, TLC, Monocyte, Platelets count, Lymphocytes Show different value having also difference in their mean  $\pm$  standard deviation value when compared to control group.

Jayaprakash and Shettu reported, changes in the haematology of fresh water grass carp exposed to low 0.075 mg/l and high 0.15 mg/l bisphenols for 15,30 and 40 days. MCV and WBC significantly increased after the exposure period, while RBC, Hb, PCV, MCH and MCHC values decreased. The results support the present study. They proposed that reduced Hb, TLC and PCV values lead to anemia due to impaired iron absorption. Stress factors can causes changes in blood

#### parameter.

Changes in erythrocyte profile indicate in vivo compensation for hypoxia due to gill damage, the nature of the change indicates release of erythrocytes from blood depots [10].

Increase in MCV and MCH value after exposure to bisphenols indicate a decrease in RBC count, which may be due to red blood cell destruction or red blood cell reduction. Leukocytes are involved in the regulation of immune function, and their numbers increase in response to the fish protective response to stress. This increase in Total Leukocytes Count (TLC) occurs due to lymphocytosis and increased release of lymphocytes from lymphoid tissue [11].

### Acknowledgment

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#### **Conflict of Interest**

All the authors confirmed that the content of this manuscript has no conflict of interest.

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