The Scientific Temper
Vol. 12, No 1-2, January-July, 2021:pp 43-47
ISSN 0976 8653, E-ISSN 2231 6396
A Web of Science Journal
Doc ID: https://connectjournals.com/03960.2021.12.43

Bisphenol-A Induced Changes in Blood Indices of *Channa punctatus* and Alleviation with Vitamin C

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ABSTRACT

Bisphenol A, a suspected endocrine disrupting chemical, is widely used in the manufacture of polycarbonate plastics and epoxy resins. With increasing industrialisation, the release of Bisphenol A in the environment is increasing in India. Bisphenol A is released in the environment from landfill leachates, industrial point sources, municipal sewage and by burning. Water bodies are the ultimate sink for Bisphenol A. People consuming fish captured from water bodies polluted with Bisphenol A can be exposed to the chemical through the fish. *Channa punctatus,* is an important food fish of India and neighbouring areas. In this study the effect of 30 days Bisphenol A exposure on the haematological parameters of *Channa punctatus* was assessed. Vitamin C, a known antioxidant, was shown to improve the changes in almost all haematological indices tested except at the highest concentrations of Bisphenol A used in this study. This study shows that *Channa punctatus* can be used as a useful sentinel species to assess pollution of water bodies by Bisphenol A and that the deleterious effects of Bisphenol A are probably caused by increasing oxidative stress in tissues which can be offset by vitamin C.

Keywords: *Channa punctatus*, Bisphenol A, BPA, Haematology, Vitamin C

INTRODUCTION

Industrialisation has led to the release of large numbers and large quantities of chemicals and synthetic agents into the environment. Amongst these, there is growing interest in the health risks posed by endocrine disrupting chemicals (EDCs). EDCs are synthetic chemicals that when absorbed in the bodies of living organisms can mimic the action of hormones, block the normal functioning of hormones, change the levels of hormones in blood and/or impact the way the body responds to the hormones. Thus, EDCs tend to disrupt the normal physiological functions of the body (Colborn et al., 1993; Diamanti-Kandarakis et al., 2009; Schug et al., 2011).

Bisphenol A (BPA) is an EDC that has been researched extensively in recent years. *In vitro* and *in vivo* studies provide convincing evidence of BPA's estrogen mimicking ability (vom Saal et al., 2006). BPA can also interfere with hormonal action by binding to thyroid and androgen receptors in the body (Nagel and Bromfield, 2013). BPA is widely used in the manufacture of polycarbonate plastics, epoxy resins, thermal printer paper and dental sealants. A significant quantity of BPA is released into the environment every year. Thus, exposure to BPA is widespread and far exceeds the body's detoxification mechanisms. BPA in industrial effluents, household sewage and landfill leachates ultimately reaches water bodies and pollutes them.

Aquatic organisms such as fish play a fundamental role in the ecosystem. The continuous release of industrial chemicals into the environment and the role of aquatic ecosystems as the ultimate sink for these xenobiotics have led to a number of toxicological studies pertaining to the effect of these anthropogenic chemicals on fish species (Arnot and Gobas, 2006). While a number of studies have investigated the effect of BPA on fish species only a few of these have been carried out in India (Lalwani et al., 2020).

In this study we chose *Channa punctatus*, a common fresh water edible fish of India and neighbouring regions as the animal model. Owing to its hardy nature and availability throughout the year, *C. punctatus* is a popular laboratory animal for toxicological studies (Nangia, 2020a).

Fish blood parameters are reliable tools for identification of stress caused by chemical stressors (Roche and Boge, 1996; Fazio, 2019). Blood parameters can be used effectively in monitoring the response of fish to toxicants in their habitat and in investigating sublethal effects of pollutants (Gaber et al., 2013; Maurya et al. 2019). Changes in Red Blood Cells (RBC) count, haemoglobin (Hb) levels, hematocrit etc. are widely employed in toxicological studies to understand the detrimental effects of xenobiotics on fish health. Bisphenol A exposure is known to induce oxidative stress in organisms. Vitamin C is a potent antioxidant. Several studies have shown that owing to its antioxidant properties, co-treatment with vitamin C can alleviate the toxicological response in living organisms (Binjhade and Shrivastava, 2012; Murmu and Shrivastava, 2014). In this study we estimated the changes induced in haematological indices in *C. punctatus* exposed to sublethal concentrations of BPA for 30 days. The ability of vitamin C to offset the changes brought about by BPA was also investigated.

MATERIALS AND METHODS

Fish were procured from Sumera reservoir in Aligarh (Nangia, 2020b) and transported to the Toxicology laboratory, D.S. College, Aligarh. The fish were measured, weighed and treated with 0.04% KMnO4 solution for two minutes (Kumar et al., 2010) to treat them for dermal infections, if any. Apparently healthy and injury free fish were used for the study. Fish were maintained according to the guidelines of APHA (1998). During the entire course of the study, the following range of water parameters was maintained: temperature 25.0 ± 1.0°C, pH 7.4 ± 0.3, dissolved oxygen 7.5 ± 0.5 mg O_2/L, conductivity 290 ± 20 µS/cm and hardness 180 ± 5 mg/L.

The 96 hours LC50 of BPA to *Channa punctatus* was determined by the Probit analysis method (Finney, 1971) to be 12.3 mg/L. Three different sublethal concentrations of BPA were chosen for this study. These were 10% (1.23 mg/L), 20% (2.46 mg/L) and 30% (3.69 mg/L) of the 96 hours LC50 value.

For antioxidant exposure, 50 mg/L vitamin C was added to the aquarium water of 4 test groups. The concentration and method of antioxidant exposure were based on previous reports (Murmu and Shrivastava, 2014).

Fish were divided into 9 groups for the study. Groups I, II and III were the negative control, solvent control and vitamin C control, respectively. Groups IV, VI and VIII were exposed to 10% (1.23 mg/L), 20% (2.46 mg/L) and 30% (3.69 mg/L) of 96 h LC50 of BPA. Groups V, VII and IX were co-exposed to 50 mg/L vitamin C along with the aforementioned concentrations of BPA i.e., 10%, 20% and 30% of 96 h LC50, respectively. The exposure was continued for 30 days using a semi-static renewal system. At the end of 30 days, fish were collected with a dip net and anaesthetised using benzocaine (Nangia, 2020b). Blood was collected from the caudal vein using an EDTA rinsed syringe and used for haematological studies. The total erythrocyte count and total leucocyte count were estimated using Neubauer Haemocytometer (Dacie and Lewis, 1975). Haemoglobin (Hb) concentration of fish blood was estimated by Sahli's acid haematin method as described by Chakrvarthy and Dierolf (2010). ESR and haematocrit% were determined by the method of Wintrobe and Landberg (1935) as described by Chakrvarthy and Dierolf (2010).

Table-1: Changes in Haematological Parameters of *C. punctatus* after Exposure to BPA alone and after Co-exposure to BPA and Vitamin C for 30 days

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	Group IX
RBC Count ($x10^6/mm^3$)	4.44± 0.13 ^{a}	4.42± 0.07 ^{a}	4.57± 0.05 ^{a}	3.39± 0.14 ^{b1p}	4.13± 0.06^{aq}	3.03± 0.07 ^{b1p}	3.95± 0.26^{aq}	2.85± 0.12 ^{b1p}	3.51± 0.14^{bq}
Haemoglobin (g/dL)	12.45± 0.35 ^{a}	12.61± 0.22 ^{a}	13.25± 0.70 ^{a}	8.97± 0.36 ^{b1p}	11.60± 0.50^{aq}	7.94± 0.32 ^{b1p}	10.74± 0.72^{aq}	7.44± 0.43 ^{b1p}	9.80± 0.75 ^{bp}
Haematocrit (%)	35.94± 1.03 ^{a}	36.96± 0.87 ^{a}	38.12± 0.96 ^{a}	27.87± 1.58 ^{b1p}	34.79± 0.40^{aq}	24.68± 0.53 ^{b1p}	32.11± 1.16^{aq}	23.87± 0.75 ^{b1p}	28.72± 0.86^{bq}
ESR (mm/ hour)	5.02± 0.22 ^{a}	5.11± 0.12 ^{a}	4.83± 0.12 ^{a}	6.63± 0.19 ^{b1p}	5.31± 0.17^{aq}	7.20± 0.20^{b12p}	5.83± 0.15^{aq}	8.02± 0.21 ^{b2p}	6.40± 0.15^{bq}
WBC Count ($x10^3/mm^3$)	55.50± 0.73 ^{a}	57.02± 0.75 ^{a}	55.86± 0.81 ^{a}	61.07± 0.63 ^{b1p}	56.56± 0.86^{aq}	67.05± 1.52^{b12p}	57.56± 0.78^{aq}	70.87± 0.97 ^{b2p}	60.59± 0.81^{bq}

Values are mean ± SEM of three replicates. Values with different letter (a, b) superscripts differ significantly ($p \leq 0.05$) with respect to control. Values with different numerical (1, 2) superscripts show significant dose dependent difference ($p \leq 0.05$) between groups IV, VI and VIII. Values with different letter (p, q) superscripts show significant difference ($p \leq 0.05$) between BPA alone (Groups IV, VI and VIII) and corresponding BPA + vitamin C co-exposure group (Groups V, VII and IX) as analysed by one way ANOVA followed by Tukey's post hoc analysis.

RESULTS AND DISCUSSION

BPA exposure of *C. punctatus* for 30 days resulted in a significant decline in RBC count, haemoglobin percentage and haematocrit. There was a concomitant, dose-dependent increase in ESR and WBC count after BPA exposure. ESR and TLC showed a significant dose-dependent change in after BPA exposure. Fish that were co-exposed to both BPA and vitamin C showed a significant improvement in all the haematological parameters compared to fish exposed to BPA alone. Co-exposure to vitamin C offset BPA induced changes in haematological parameters in all groups resulting in values comparable to Group I. However, group IX fish i.e., fish co-exposed to vitamin C and the highest concentration of BPA tested in this study. showed a significant change in all parameters compared to the control group. The results are summarised in Table 1.

A reduction in RBC count after BPA exposure has been reported in Channa striatus (Elvin et al., 2020), Labeo bata (Mukherjee et al., 2020), albino mice (Sujan et al., 2020) etc. The reduction in RBC count could be due to reduced erythropoiesis or increased haemolysis of RBCs (Witeska, 2013). BPA is also reported to interfere with the synthesis of haemoglobin (Krishnapriya et al., 2017). In fish, the kidney is the principal haematopoietic organ. BPA exposure is reported to cause degeneration and necrosis of renal haematopoietic tissue in *Catla catla* (Faheem et al., 2016). Haemolysis after BPA treatment has been reported by Sangai et al. (2018) and Vaidya et al. (2019). Both haemolysis and degeneration of renal tissue has been reported in *Gambusia affinis* and *Poecilia reticulata* (Elshaer et al., 2013).

Though the exact mechanism of BPA toxicity is uncertain, some studies suggest that BPA toxicity could be due to the induction of reactive oxygen species (ROS) and consequent oxidative stress (Rochester, 2013). Sangai et al. (2018) have suggested that ROS react with and destabilize the membrane of RBCs resulting in an influx of water and consequent haemolysis. The increase in WBC count after BPA exposure is consistent with the findings in *Perca flavescens* (Rogers and Mirza, 2013) and *Pseudoetroplus maculatus* (Asifa and Chitra, 2018). The increase in WBC count is suggestive of toxicant induced tissue and cellular damage such as necrosis which in turn could trigger a non-specific immune response (Das and Mukherjee, 2003).

The protective role of vitamin C on toxicant induced alteration of haematological parameters has been reported by Narra et al. (2015) and Hounkpatin et al. (2012). The ameliorative effect of vitamin C on haematological parameters is probably due to its antioxidant nature (Binjhade and Shrivastava, 2012). Fish in group IX, which received the highest dose of BPA with vitamin C, did not show a complete recovery in various parameters after 30 days exposure. This suggests that vitamin C cannot mitigate the extensive damage caused by prolonged exposure to very high concentration of BPA.

ACKNOWLEDGEMENTS

The authors are thankful to the Principal, Dharam Samaj College, Aligarh for permitting the research and for providing necessary laboratory facilities. The authors are also thankful to Dr. B.R.A. University, Agra for facilitating the study and arranging necessary guidance.

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