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Effect of Bisphenol-A Exposure on Activity of Antioxidant Enzymes in *Channa punctatus* and Alleviation with Vitamin C

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ABSTRACT

Bisphenol-A (BPA) is a high volume industrial chemical that is widely employed in the manufacture of polycarbonate plastics, epoxy resins, flame retardants and other industrial products. The release of BPA into the environment far exceeds its removal from the different environmental matrices. Alarming levels of BPA have been reported in air, water, soil and biological fluids like serum, urine and even amniotic fluid. As waterbodies serve as the ultimate sink for toxicants, the effect of BPA on aquatic fauna needs to be investigated. BPA and other similar xenobiotics are known to induce oxidative stress by disturbing the redox balance of the body. In this study we looked into the activity of antioxidant enzymes of the liver in *Channa punctatus* following BPA exposure. As vitamins are also known to reduce oxidative stress, the effect of co-exposure of vitamin C and BPA was also studied. We observed significant changes in the activity of antioxidant enzymes as a result of BPA exposure and alleviation of these effects due to vitamin C.

Keywords: BPA, oxidative stress, SOD, Catalase, GST, antioxidant, vitamin C

INTRODUCTION

Bisphenol A (BPA) is an organic compound that is extensively used in the manufacture of polycarbonate plastics, epoxy polymers, flame retardants and thermal printer paper (Geens et al., 2011). BPA is produced by condensation of two parts phenol with one part acetone (Vautherin and Cardoso, 2019). BPA based compounds and plastics are employed in making a vast array of products. It is no surprise therefore that BPA production and consumption is increasing at a tremendous pace. As per the global BPA market report in 2018, BPA annual production is expected to reach to 7348 thousand tonnes by the year 2023 (Naomi et al., 2022). BPA is reported to leach from plastic wastes (Yamamoto and Yasuhara, 1999) and epoxy resins (Howdeshell et al., 2003). The presence of BPA has been reported in all environmental matrices i.e. air, water and soil, being especially worrisome in waterbodies as they serve as the ultimate sink for toxicants.

If the rate of generation of reactive oxygen species (ROS) exceeds the rate at which the physiological antioxidant mechanisms remove them, it results in oxidative stress (Sies, 1986). The antioxidant defences of animals include enzymes like catalase (CAT), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione-S-Transferase (GST) etc. Several studies highlight the induction of oxidative stress in animals following BPA exposure (Qiu et al, 2016, Gu et al., 2020). Several studies have shown that BPA exposure results in changes in the activity of antioxidant enzymes (Faheem et al., 2020; Zhang et al., 2020).

Non enzymatic antioxidants like vitamin C and vitamin E also play an important role in the deactivation of free radicals (Sies, 1997). Vitamin C or ascorbic acid is a naturally occurring water soluble vitamin that is extremely effective as an antioxidant agent (Bendich et al., 1986) and alleviates toxicant induced oxidative stress in fish (Abdelazim et al., 2018; Fathima et al., 2019).

Table 1: Activity of Antioxidant Enzymes in the Liver of *C. punctatus* after Treatment

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII	Group IX
SOD (U/mg protein)	21.67 ± 1.77 [a]	20.19 ± 1.48 [a]	22.82 ± 0.89 [a]	20.51 ± 1.82 [a1p]	21.75 ± 1.56 [ap]	18.28 ± 1.68 [a1p]	21.03 ± 1.89 [ap]	13.31 ± 0.91 [b1p]	20.90 ± 1.83 [ap]
CAT (U/mg protein)	130.53 ± 3.23 [a]	129.17 ± 3.55 [a]	135.08 ± 3.64 [a]	119.46 ± 3.20 [a1p]	127.16 ± 3.36 [ap]	110.75 ± 3.17 [b1p]	127.41 ± 3.20 [aq]	108.66 ± 3.32 [b1p]	125.89 ± 3.36 [aq]
GPx (U/mg protein)	47.56 ± 3.12 [a]	46.37 ± 2.13 [a]	52.77 ± 3.40 [a]	40.40 ± 4.40 [a1p]	42.42 ± 3.19 [ap]	29.75 ± 2.13 [b1p]	31.62 ± 3.75 [bp]	31.81 ± 2.32 [b1p]	28.92 ± 2.19 [bp]
GST (mU/mg protein)	237.77 ± 19.22 [a]	232.72 ± 16.66 [a]	221.15 ± 14.38 [a]	291.30 ± 13.37 [a1p]	238.88 ± 8.08 [ap]	335.95 ± 14.37 [b1p]	300.74 ± 14.13 [ap]	331.42 ± 14.42 [b1p]	301.76 ± 10.80 [ap]

Note: *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.*

Fish are highly suited for toxicological studies because they are exposed to the toxicant through their entire body and can be easily cultured in the laboratory to study the effect of any particular toxicant. *Channa punctatus*, the snake headed murrel, is a hardy fish and a popular model organism for toxicological studies (Yadav and Trivedi, 2009; Sharma et al., 2019). In this study we investigated the effect of BPA on the activity of liver antioxidant enzymes and the ameliorative effect of co-exposure to vitamin C.

MATERIALS AND METHODS

C. punctatus of length 12 ± 2 cm and weight 22 ± 3 g were used in this study. The fish were procured from Sumera reservoir, Aligarh from where they were carefully transported to the toxicology laboratory, D.S. College, Aligarh to avoid injury and stress to the animals during transportation. Following prophylactic treatment with 0.02% KMNO4, the fish were allowed to acclimatize to laboratory conditions for two weeks as per APHA guidelines (APHA, 1998). Throughout the study, natural photoperiod was maintained and fish were fed at 12 hours intervals with commercial fish feed. Temperature (23-25^{o}C), pH (7.6-7.8), dissolved oxygen (7.2-7.6 mg/L), hardness (175-185mg/L) and conductivity (270-300 µS/cm) were monitored during the entire study as per APHA guidelines (APHA, 1998).

A 20% stock solution of BPA (HiMedia Laboratories Pvt. Ltd, Mumbai, India) was prepared in 100% analytical grade ethyl alcohol (Nangia 2020; Nangia and Yadav, 2021). Appropriate dilutions of the stock solution were used for treatment. Three different concentrations of BPA i.e. 10 %, 20 % and 30 % of 96 hours LC50 as reported in a previous study from our lab were used (Yadav and Nangia, 2021). Based on previously published data the concentration of vitamin C chosen for the study was 50mg/L (Murmu and Shrivastava, 2011). Both vitamin C and BPA were added to the aquarium water.

The fish were divided into 9 treatment groups. Six acclimatized fish were randomly assigned to each group and treated for 15 days. Group I, II and III served as control, solvent control and vitamin C control. Group IV, VI and VIII were the BPA exposure groups. These received 1.23 mg/L, 2.46 mg/L and 3.69 mg/L of BPA, respectively. Groups V, VII and IX were the BPA and vitamin C co-exposure groups. The treatment was continued for 15 days.

At the end of the treatment period the fish were collected gently with a dip net and anaesthetized using benzocaine solution (50 mg/L). Liver of the fish was dissected out and used for estimation of antioxidant enzyme activity. Superoxide dismutase (SOD) activity was estimated by the modified formazan inhibition method of Kakkar et al. (1984). Catalase (CAT) activity was estimated by the method of Sinha (1972). To estimate glutathione peroxidase (GPx) activity the method of Lawrence and Burk (1976) was used. Glutathione-S-Transferase was estimated by the method of Habig et al. (1974).

RESULTS AND DISCUSSION

The activity of different antioxidant enzymes was estimated in the liver of *Channa punctatus* after 15 days treatment with 3 different BPA concentrations and co-exposure with vitamin C. These results have been summarized in Table 1.

SOD activity in liver

After 15 days of exposure to BPA, the superoxide dismutase activity in the liver of control fish (group I) was 21.67 ±

1.77 U/mg protein and there was no significant difference between the three control groups. After BPA exposure, SOD activity declined; the decrease being significant only in group VIII. When *C. punctatus* were exposed to BPA with vitamin C there was no significant change in SOD activity in any vitamin C co-exposure group with respect to the groups that had been exposed to the corresponding concentration of BPA alone for 15 days (groups IV, VI and VIII).

Catalase activity in liver

After 15 days the catalase activity in the liver of control fish (group I) was 130.53 ± 3.23 U/mg protein. There was a decrease in catalase activity in groups exposed to BPA alone. When *C. punctatus* were exposed to BPA with vitamin C catalase activity in groups VII and IX was significantly more than the corresponding BPA only exposure groups (groups VI and VIII, respectively).

GPx activity in liver

There was no significant difference in the liver GPx activity of the three control groups. In groups exposed to BPA a significant decline in GPx activity was observed in groups VI and VIII. Though the GPx activity in the three groups co-exposed to BPA and vitamin C tended to be higher compared to groups exposed to BPA alone, there was no statistically significant difference between them.

GST activity in liver

After 15 days of exposure to different concentrations of BPA, liver GST activity in groups VI and VIII were significantly higher than that in the 15 days control group. GST activity in groups exposed to BPA and vitamin C together for 15 days showed no significant difference vis-a-vis BPA alone exposure groups.

Quesnot et al. (2014) reported that BPA is capable of inhibiting enzymes of phase I and phase II biotransformation pathways. They also determined that phase II transformation was responsible for handling more than 90 % of all BPA metabolites in the body. The liver is the primary organ for detoxification of xenobiotics (Ferreira et al., 2014). In goldfish, it is also the organ with the highest concentration of BPA (Rouleau and Kohli, 2008). Hassan et al. (2012) reported that BPA exposure causes hepatotoxicity by increasing oxidative stress. BPA exposure has been reported to induce alterations in the markers of oxidative stress in different fish species like *Cyprinus carpio* (Qiu et al., 2016; Gu et al., 2020), *Labeo rohita* (Faheem et al., 2020), *Oreochromis mossambicus* (Chitra and Maiby, 2014) and *Oryzias latipes* (Wu et al., 2011). In the present study, BPA exposure resulted in changes in the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-s-transferase (GST).

The results suggest that BPA exposure inhibited the activity of SOD, catalase and GPx. This in turn may result in increased lipid peroxidation in liver tissue of *Chana punctatus*. Increased lipid peroxidation in turn may lead to liver damage (Yadav and Nangia, 2021). These observations are supported by several studies. Prasanth and Sadasivan (2013) reported that BPA could bind to the active site of human SOD and inhibit its catalytic activity. Similarly, Leem et al. (2016) reported that in human bone marrow stem cells, BPA exposure caused an overload of superoxide anion resulting in lipid peroxidation and consequent cell death.

Jayakanthan et al. (2015) reported that BPA binds to catalase. Piao et al. (2019) reported that binding of BPA could reduce catalase activity by upto 29.1%. In this study, there was a reduction in GPx activity after BPA exposure. These results are supported the work of Faheem et al. (2020) in *Labeo rohita* larvae after 21 days, Srivastava and Reddy (2019) in *H. fossilis* after 28 days, Abdel-Tawwab and Hamed (2018) in *Oreochromis niloticus* after 6 weeks, Qiu et al. (2016) in *Cyprinus carpio* after 30 days and Deepa et al. (2015) in *Oreochromis mossambicus* after 28 days. The increase in GST activity after BPA exposure observed in this study is supported by the reports of Yang et al. (2013), Deepa et al. (2015), Li et al. (2016), Kaya and Kaptaner (2016) and Faheem et al. (2020). We propose that the increase in activity of GST after BPA exposure is because GST is an important phase II biotransformation enzyme (Elegbede et al., 1993) and phase II reactions are important in the biotransformation of BPA (Michałowicz, 2014). However, Maradonna et al. (2014) did not find any change in GST activity after BPA exposure. The variations in GST activity after BPA exposure may be due to the differences in dose of BPA, animal model or route of administration.

The amelioration of the deleterious effects of BPA exposure may be because vitamin C is a known scavenger of ROS like singlet oxygen, superoxide radical, hydroxyl radical etc. (Frei et al., 1989; Pehlivan, 2017). Secondly, though vitamin C is not lipid soluble it regenerates vitamin E which in turn plays an important role in terminating the lipid peroxidation chain reaction (Benzie, 1996).

The results of this study suggest that homeostatic mechanisms of *Channa punctatus* may be capable of handling the chemical insult due to low ambient concentration of BPA. This is why low BPA concentrations do not trigger a statistically significant change in antioxidant response in *Channa punctatus.* This study also suggests that activity of antioxidant enzymes in the liver of C. punctatus may be a useful biomarker for assessing oxidative stress due to environmental toxicants.

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