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Original Article

# Dose Dependent Effects of Bisphenol A Exposure on Locomotor Activity, Acetylcholinesterase and Redox System Parameters in Zebrafish Embryos

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**Abstract**

**Introduction:** Endocrine disrupting chemicals (EDC) are either synthetic or natural compounds in the environment that can interfere with endocrine functions. Exposure to EDCs during development is a major concern, and the health consequences may be permanent or long-lasting. Bisphenol A (BPA) is known to be an EDC and prenatal BPA exposure has been related to differences in children's brain microstructure, leading to differences in children's behavioral symptoms. Moreover high BPA exposure during pregnancy is related to increased behavioral problems throughout childhood. In our study we aimed to evaluate the effects of BPA exposure in zebrafish embriyos focusing on locomotor activities and biochemical parameters.

**Methods:** Zebrafish embryos were exposed to 1µg/L and 10 µg/L BPA until 72 hpf. At the end of exposure period, locomotor activities were determined and acetylcholinesterase (AChE), glutathione S-transferase (GST) and superoxide dismutase (SOD) activities were determined using spectrophotometric methods.

**Results:** Concentration dependent changes were determined in GST and SOD activites indicating increased response to oxidative stress due to BPA toxicity. AChE acitivity alterations and locomotor activity changes pointed out the importance of concentration in the neurotoxic effects of BPA in zebrafish embryos.

**Conclusion:** The results of our study pointed out that new studies are needed to examine the effects of BPA, especially on cognitive and locomotor functions.

**Keywords:** Bisphenol A, locomotor activity, oxidative stress, acetylcholinesterase, zebrafish embryos

## 1. Introduction

Bisphenol A (BPA) is a chemical which is manufactured in large amounts for use principally in the production of polycarbonate plastics. It can be found in a variety of items, such as shatterproof glass, eyewear, water containers, and epoxy resins used to coat some metal food containers, bottle caps, and water supply piping. For the majority of people, eating habits are the main way they are exposed to BPA. Although additional potential exposure sources like air, dust, and water exist, the vast majority of daily human contact with BPA comes from food and drinks (1,2).

BPA is associated with harmful health effects in mammalian and non-mammalian systems, ecosystems, and in vitro models, according to a substantial body of evidence obtained from more than 300 published research. As a well-known endocrine disruptor BPA binds to estrogen receptors and has estrogenic effects (1). BPA is also referred as a popular model for illustrating the low dose and unconventional characteristics of hormones and endocrine disruptors that control or alter the endocrine system.

The cholinergic enzyme acetylcholinesterase (AChE) is mainly present in postsynaptic neuromuscular junctions, particularly in muscles and nerves. Acetylcholine (ACh), a neurotransmitter that occurs naturally, is instantly hydrolyzed into acetic acid and choline. AChE's main function is to halt synaptic transmission and messaging between neurons in order to stop ACh from spreading and activating adjacent receptors. AChE is inhibited by organophosphates. They play a significant role in the creation of insecticides and nerve agents (3). Cholinergic neurons in the brain regulate reception of stimuli, thinking, and awareness. Cholinergic neurons not only cover the forebrain, but also the brainstem and thalamus, including the reticular nucleus, which control cognition and attention. These cholinergic neurons have damaged projections in the setting of Alzheimer dementia as a degenerative illness, which correlate with the traditional signs of cognitive slowness and deterioration. Short-term memory loss, cerebrum atrophy, B-amyloid plaques, tangles, and tau protein deposits are all common symptoms of the condition (4).

In recent years BPA exposure has been shown to increase the risk of developing neurodevelopmental, neurodegenerative diseases including Alzheimer's and Parkinson's diseases (5). In line with this information, in our study, the effect of BPA exposure on locomotor activity and the possible relationship between this effect and AChE activity were investigated in zebrafish embryos. Antioxidant enzyme activities were also determined to determine the response to BPA toxicity.

## 2. Methods

### 2.1. Embryo exposure and determination of locomotor activity

Zebrafish embryos were exposed to BPA (5 µg/L and 10 µg/L) in well plates for 72 hours post-fertilization (hpf). The embryo medium eas used as blank control. For the biochemical analyses 50 embryos/pool and 3 biological replicates for each group was prepared. Exposure solutions were renewed with fresh solutions each day and the developmental parameters were investigated using a stereomicroscope (Zeiss Discovery V8, Germany). The locomotor activity of the zebrafish embryos at 72 hpf was evaluated described previously (6).

To accomplish this, a 60 mm Petri dish filled with embryo media was placed on top of the motility wheel, which is mounted on the microscope stage. The zebrafish embryo was then placed in the center of the motility wheel using an embryo poker tool, the length of time it takes for an embryo to swim that distance was recorded, and the average escape reaction was computed. Zebrafish embryos at 96 hpf have been immobilized at the conclusion of the exposure period by immersion in freezing water for 10 minutes, followed by five minutes of sodium hypochlorite, to assure death. No ethical permission was necessary for the techniques utilized because the zebrafish embryos used were no older than 5 days old, as indicated by the Council of Europe in 1986, Directive 86/609/EEC.

### 2.2. Determination of total protein

The concentrations of total proteins in the samples were assessed using Lowry's technique (7). In this procedure, proteins are first reduced by the Folin reagent before reacting with copper ions in an alkali media. At 500 nm, the absorbances are calculated. The values for each protein were computed and presented using the total protein levels.

### 2.3. Determination of Acetylcholinesterase activity

The supernatants were examined for acetylcholinesterase (AChE) activity using the method of Ellman (8). In this procedure, acetylcholinesterase produces thiocholine, which reacts with 5,5'-dithiobis (2-nitrobenzoic acid) to generate a yellow tint. The enzyme activity in the sample is determined by measuring the magnitude of the yellow product color at 412 nm.

### 2.4. Determination of Superoxide dismutase

The method used to assess superoxide dismutase (SOD) activity is based on SOD's capacity to enhance the effects of riboflavin-sensitized photo-oxidation of o-dianisidine. By shining a fluorescent lamp on the reaction mixture made up of riboflavin and O-dianisidine dihydrochloride, superoxide activity is created. Riboflavin sensitizes and SOD enhances the oxidation of O-dianisidine, and the enhancement is linearly correlated with SOD concentration. Using a spectrophotometer set at 460 nm, the absorbances at 0 and 8 minutes of illumination were measured, and the net absorbances were determined (9). The data were expressed as U/mg protein.

### 2.5. Determination of Glutathione-S-transferase

The evaluation of Glutathione-S-transferase activity was performed through the determination of the absorbance of the product obtained by the conjugation of GSH and 1-chloro-2,4-dinitro-benzene (CDNB) through spectrophotometric analysis at 340 nm (10).

## 3. Results

When compared with the control group, average speed and distance travelled decreased significantly in the 1 µg/L BPA exposed group (****p<0.0001). On the other hand, no significant change in average speed was observed in the 10 µg/L BPA exposed group while distance travelled decreased significantly in the same group (*p<0.05). Moreover, average speed and distance travelled levels of the 10 µg/L BPA exposed group was significantly higher than the 1 µg/L BPA exposed group (****p<0.0001) (Fig 1 and 2).

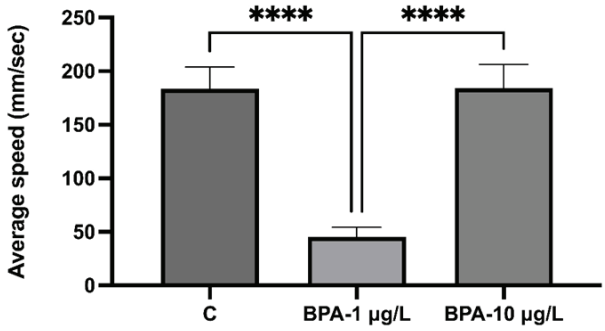


**Figure 1.** Average speed results of the embryos in in the control and BPA groups. *Data presented are mean ± SD. ****p<0.0001, SD: standard deviation.*

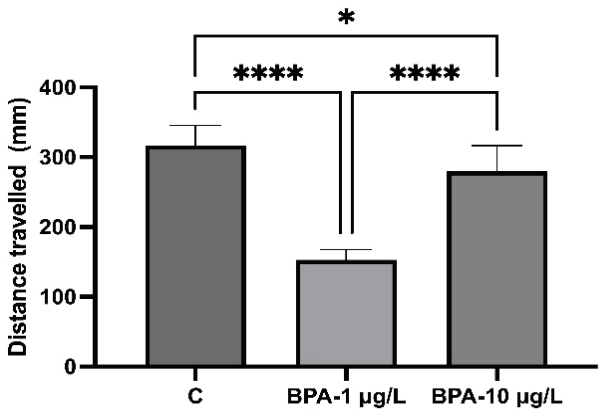


**Figure 2.** Total distance results of the embryos in in the control and BPA groups. *Data presented are mean ± SD. ****p<0.0001, * p<0.05. SD: standard deviation.*

AChE activity increased significantly both in the 1 µg/L and 10 µg/L BPA exposed groups (**p<0.01 and ****p<0.0001 respectively). AChE activity of the 10 µg/L BPA exposed group was significantly

higher than the 1 µg/L BPA exposed group (****p<0.0001) (Fig 3).

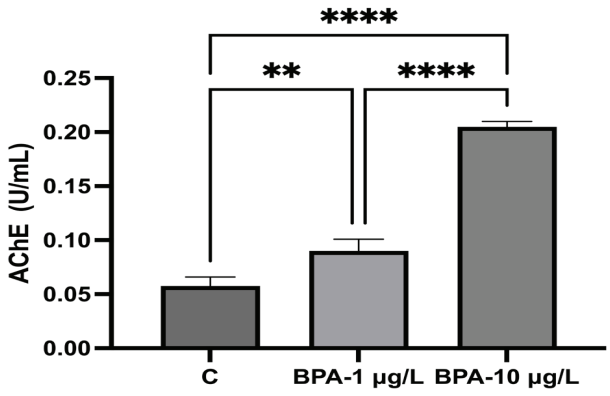


**Figure 3.** AChE activities of the embryos in the control and BPA groups. *Data are expressed as mean+SD from the three independent experiments. ****p<0.0001, ** p<0.01, SD: standard deviation.*

SOD activity decreased significantly both in the 1 µg/L and 10 µg/L BPA exposed groups (***p<0.001 and *p<0.05 respectively). SOD activity of the 10 µg/L BPA exposed group was significantly higher than the 1 µg/L BPA exposed group (*p<0.05) (Fig 4).

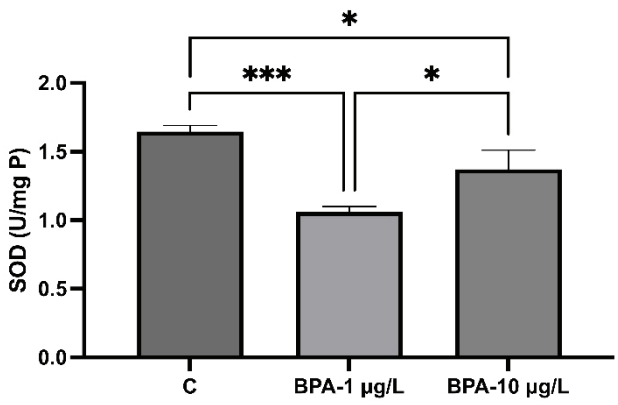


**Figure 4.** SOD activities of the embryos in the control and BPA groups. *Data are expressed as mean+SD from the three independent experiments. *** p<0.001, * p<0.05. SD: standard deviation.*

GST activity increased significantly both in the 1 µg/L and 10 µg/L BPA exposed groups (*p<0.05 and ***p<0.001 respectively). Moreover, GST activity of the 10 µg/L BPA exposed group was significantly higher than the 1 µg/L BPA exposed group (*p<0.05) (Fig 5).

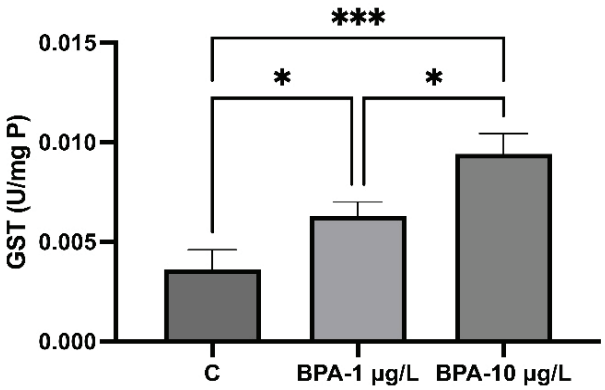


**Figure 5.** GST activities of the embryos in the control and BPA groups. *Data are expressed as mean+SD from the three independent experiments. *** p<0.001, * p<0.05. SD: standard deviation.*

## 4. Discussion

Results of our study showed that BPA caused dose dependent changes in locomotor activity in zebrafish embryos. Previous studies showed that BPA induced abnormalities in nonreproductive behaviors in rodents including locomotor activity, spontaneous motor activity, and aggressive behavior. Nojima et al., evaluated the effects of BPA on the spontaneous motor activity of adult male rats and reported that BPA induced hyperactivity in adult male rats (11).

In accordance with our results regarding the increased the locomotor activities, in male rats postnatal BPA exposure was found to increase the spontaneous motor activity. However, BPA exposure during perinatal and postnatal stages increased the locomotor activities in 1 month old female mice while it decreased it in the male mice of same age (12). According to the results of these studies BPA was shown to pass through blood −brain barrier and alter the functions of brain in adult rats. On the other hand, how intraperitoneally administered BPA affects brain and induce hyperactivity in adult is suggested to need further investigation (11).

In our study, AChE activity increased both in the 1 µL and 10 µL BPA groups,.The alterations in the AChE activities might be associated with the altered locomotor activity.

Xuereb et al., investigated the relation between AChE inhibition and the alterations in feeding and locomotor activity in male Gammarus fossarum exposed to chlorpyrifos which is an organophosphorous pesticide and carbamate pesticide methomyl (MT) for 96 hours (13). They reported reduced AChE activity in a concentration-dependent way and also altered behavioural parameters in both CPE and MT exposed groups. With regard to both the rate of feeding and locomotor behavior—both of which are recognized as important ecological responses—this study offers a basis for interpreting the biomarker AChE at the higher biological organisation stage.

In various laboratory models as well as in humans, oxidative stress and related indicators are linked to BPA toxicity. There is growing evidence that BPA organ toxicity is greatly influenced by the generation of reactive oxygen species (ROS) and/or a diminished ability of antioxidant defense, which changes the oxidative equilibrium in the mitochondria and throughout the cell (14). The effects of BPA on increasing oxidative stress in zebrafish and zebrafish embryos have been demonstrated in previous studies (15,16).

In our study SOD activity decreased in the 1 µg/L and 10 µg/L BPA groups. Increased activity of SOD may indicate the activation of the antioxidant defence mechanism in response to increased oxidative stress. On the other hand, GST activities increased significantly in the BPA exposed groups in a dose dependent pattern. Increased GST activity may also be regarded as a defence mechanism in case of increased oxidative stress due to BPA toxicity as GST plays a major role in the detoxification process. Similar to the results of our study, BPA has also been shown to induce oxidative stress which was decreased by antioxidant enzyme activities, showing the protective mechanism against oxidative stress. Moreover BPA is reported to be more toxic at the early stages of the embryonic development (17).

## 5. Conclusion

As a conclusion results of our study showed dose dependent alterations in the locomotor activities in BPA exposed zebrafish embryos. Increased antioxidant system parameters indicated the oxidant-antioxidant balance that was disturbed by BPA exposure. This finding supports the results of previous studies on BPA. We think that the changes observed in the form of stimulation of locomotor activity in our study may be associated with changes in AChE activity, which is closely related to cognitive functions. The results of our study pointed out that new studies are needed to examine the effects of BPA, especially on cognitive and locomotor functions.

**Conflicts of interest:** The authors report no conflicts of interest.

**Ethics approval:** As the zebrafish embryos used were no older than 5 days old, no ethical approval was required for the protocols applied as stated by the Council of Europe (1986), Directive 86/609/EEC.

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