Received: 6 June 2020 | Revised: 18 March 2021 | Accepted: 23 July 2021

DOI: 10.1002/jbt.22873

RESEARCH ARTICLE

WILEY

# Bisphenol A exposure induces neurobehavioral deficits and neurodegeneration through induction of oxidative stress and activated caspase-3 expression in zebrafish brain

Pradyumna K. Sahoo[1] | Sai Aparna[2] | Pradeep K. Naik[3] | Shashi B. Singh[4] | Saroj K. Das[1]

[1]Neurobiology Laboratory, Centre for Biotechnology, Siksha 'O' Anusandhan (Deemed to be University), Kalinga Nagar, Bhubaneswar, India

[2]Neurobiology Laboratory, Department of Zoology, Ravenshaw University, Cuttack, India

[3]Department of Biotechnology and Bioinformatics, Sambalpur University, Burla, India

[4]National Institute of Pharmaceutical Education and Research (NIPER), Balanagar, Hyderabad, India

**Correspondence**
Saroj K. Das, Neurobiology Laboratory, Centre for Biotechnology, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar 751003, India.
Email: sarojkumardas@hotmail.com and sarojkumardas@soa.ac.in

## Abstract

Bisphenol A (BPA) is noted for its adversative effects by inducing oxidative stress, carcinogenicity, neurotoxicity, inflammation, etc. However, the likely act of BPA in inducing neurodegenerative phenotypes remains elusive in the available literature. Hence, the present study was conducted to decipher the neurodegenerative potential of BPA in inducing Parkinson's disease like phenotypes in zebrafish. Zebrafish were subjected to chronic waterborne exposure to BPA for 56 days. Locomotor activities and neurobehavioral response were assessed by the NTDT (novel tank diving test), OFT (open field test), and LDPT (light-dark preference test). The oxidative stress markers and histopathological observation for pyknosis and chromatin condensation were carried out. Immunohistochemistry for activated caspase-3 and targeted proteins expression study was performed. The basic findings reveal that chronic BPA exposure significantly induces locomotor dysfunction through a significant decline in mean velocity and total distance traveled. As a measure of pyknosis and chromatin condensation, pyknotic and Hoechst positive neurons in telencephalon and diencephalon significantly increased by BPA exposure. A higher concentration of BPA adversely affects the neurobehavioral response, antioxidant status, and neuromorphology in zebrafish. Parkinson-relevant targeted protein expression viz. alpha-synuclein and LRRK2, were significantly upregulated, whereas tyrosine hydroxylase, NeuN, and Nurr1 were significantly downregulated in the zebrafish brain. As an indicator of cell death by apoptosis, the expression of activated caspase-3 was significantly increased in the BPA-exposed zebrafish brain. These basic results of the current study indicate that chronic waterborne exposure to BPA induces neuropathological manifestation leading to the development of motor dysfunction and Parkinsonism-like neurodegenerative phenotypes in zebrafish.

KEYWORDS

apoptosis, bisphenol A, locomotor impairment, neurodegeneration, neurodegenerative disease, oxidative stress

  

## 1 | INTRODUCTION

Human-caused activities have significantly augmented the level of genotoxic compounds in the soil, water, and ambient atmosphere, thus exerting the potential threat of serious health maladies, including neurodegenerative diseases. Bisphenol A (BPA) is a man-made xenoestrogen that is primarily utilized in huge quantities to yield polymers such as epoxy resins, polycarbonate plastics, and polyesters.[1] Its concentrations are generally lower than 1 μg/L in the aquatic environment; however, in industrial effluents, BPA concentration rises two to three folds.[2] BPA has been reported to induce oxidative stress, carcinogenicity, neurotoxicity, neuroinflammation, and neurobehavioral alterations.[3–10] However, the pathological manifestation involving downstream signaling is not well characterized. Earlier reports also showed the presence of BPA in humans and animals, providing conclusive evidence for potential health hazards of BPA.[11–15] Few reports also advocated that BPA has the potential to cross the blood-brain barrier (BBB) effortlessly and is accounted to have an adverse effect on the central nervous system.[16,17] BPA is also well known as an anthropogenic endocrine-disrupting chemical.[18] However, the literature on the neurotoxic potential of BPA in inducing neurodegenerative diseases is limited.

With the rising load of anthropogenic toxicants like BPA in ambient air, soil, and water, an actual risk towards development of grave health hazards may arise. The aquatic ecosystem may act as a delicate target for pollution with this xenoestrogen upsetting equally the aquatic fauna and human beings. Zebrafish (*Danio rerio*) is a perfect experimental animal presenting similar adaptive responses to toxicity tests and therapeutic approaches as that of mammals.[19,20] Currently, zebrafish is considered as a vertebrate experimental animal model to study various neurodegenerative diseases.[21] Alternatively, zebrafish are also provided with vital antioxidant enzymes at relatively higher levels than higher vertebrates.[22] Several studies have also depicted that zebrafish is retorting to mood influencing compounds in behavioral screening tests in an expected manner.[23–26] The brain, in contrast, is awfully susceptible to neurotoxicity leading to development of neurodegenerative diseases due to its weak antioxidant defense, augmented oxygen consumption rate, and high lipid content.[27,28] Consequently, a rising load of BPA potentially develops susceptibility towards development of neurodegenerative diseases in human beings. The oxidative stress induction potential of BPA might impact the brain as a soft target. Therefore, the current experimental paradigm was set to elucidate the possible role of BPA in inducing Parkinsonism-like phenotypes in zebrafish.

## 2 | MATERIALS AND METHODS

### 2.1 | Reagents and chemicals

All reagents and chemicals utilized were obtained from SRL, Sigma-Aldrich, Himedia, Bio-Rad, Invitrogen unless otherwise mentioned. The reagents used for the present study comprise BPA (#239658), Tricaine (#E10521), Glutathione (#PHR1359), DNPH (#D199303), GHCL (#G3272), TBA (#T5500) & CV (#1052350025) from Sigma-Aldrich; GSSG (#22151), NADPH (#99197), DTNB (#32363) and BHT (#82010) from SRL. The reagents used for immunoblotting and immunofluorescence studies were purchased from Bio-Rad (#1620112, #1620115, #1610737, #1610394) and Invitrogen (Hoechst #H3570, #P36935, #WP20005). The primary and secondary antibodies used for the study include α-Syn (SY SY #128002), NeuN (Abcam #Ab177487), TH (Abcam #ab229333), LRRK2 (MBS #MBS9604182), Nurr-1 (Labome #ARP38753), cleaved caspase-3 (Abcam #ab13847), actin (Abcam #ab170325), anti-mouse (Promega #W4021), anti-rabbit (Promega #W4011), goat anti-rabbit (Invitrogen # A-11036), etc.

### 2.2 | Experimental animals

All experiments mentioned were approved and performed as per the appropriate guidelines of the institutional animal ethics committee (IAEC) of Siksha 'O' Anusandhan (Deemed to be University), Odisha, India (IAEC No: IAEC/SPS/SOA/09/2020). Adult zebrafish (4–6 months old; both sexes in equal proportion) were obtained from the Central Institute of Freshwater Aquaculture (CIFA), Odisha, India, and were kept in a 40-L capacity stock aquarium and maintained as per the zebrafish standard of care.

### 2.3 | Acute toxicity test and dose standardization of BPA

A dose-response curve acute toxicity test for BPA was performed.[29] An acute toxicity test was conducted in zebrafish (seven no/group) by considering different concentrations of BPA (mg/L). Zebrafish were subjected to waterborne BPA exposure (96 h) and mortalities were recorded at 24, 48, 72, and 96 h. The waterborne acute toxicity test of BPA was conducted on zebrafish to determine $LC_{50}$. The normal laboratory condition was maintained during the test as per the acute toxicity test guideline. Different concentrations of BPA were prepared by dilution of a stock solution (0, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 mg/L). BPA solution was prepared by dissolving in 100% ethanol.

The dose-response study showed 100% death at a concentration of 8 mg/L (35.0431 μM) and is considered as $LC_{100}$[30] and the $LC_{50}$ for BPA was found to be 6 mg/L (26.2824 μM). The $LC_{50}$ was measured with increasing concentration of BPA and the mortality of zebrafish was recorded after 96 h followed by Finney's probit analysis.[31] The behavioral study (by novel tank diving test [NTDT])[32] conducted 96 h after the test, showed that behavioral paradigm-shifting (time spent in the upper zone of NTDT) started at 0.5 mg/L of BPA and continued exponentially up to 4 mg/L (17.52 μM). For the present study, two different concentrations of BPA (1 mg/L [4.38 μM] and 4 mg/L [17.52 μM]) were used to study the neurodegenerative potential of BPA in zebrafish (Please see Supporting Information data). Each of the experiments was performed as three biological repeats and the results were statistically significant.

Adult zebrafish were assigned into four groups such as naïve, control, BPA 1.0 mg/L (BPA1.0), and BPA 4.0 mg/L (BPA4.0). Forty-two zebrafish/groups were considered and kept in a 10-L aquarium. The naïve group of zebrafish was not subjected to treatment with the vehicle or solvent carrier. In the present study, the main objective for inclusion of the naïve group was to negate any possible impact of the solvent carrier or vehicle (i.e., ethanol) on the neurobehavioral response, biochemical parameters, and neuromorphology in zebrafish brain in contrast with the control group (vehicle control).

## 2.4 | Behavioral tests

### 2.4.1 | Assessment of scototaxis behavior by light/dark preference test (LDPT)

LDPT is used to study the scototaxis behavior (preference for darkness) of zebrafish.[33,34] In the current study, LDPT was carried out after 56 days of BPA exposure ($n$ = 18/group). The neurobehavioral response of individual zebrafish was video recorded and analyzed for 5 min by an ANYmaze video tracking system (Stoelting Co.).

### 2.4.2 | Evaluation of explorative and locomotory behavior by NTDT

NTDT is primarily used to evaluate exploratory and locomotory behavior in zebrafish.[32] Zebrafish preferentially showed bottom-dwelling behavior. After exposure to the stipulated time period of BPA exposure, individual zebrafish of the respective experimental group were subjected to neurobehavioral testing and analysis for 5 min with the help of an ANYmaze video tracking system ($n$ = 18/group). NTDT was also used to study the motor dysfunction with the help of the ANYmaze system through assessment of total distance traveled, mean velocity, and the number of transitions to the top zone.

### 2.4.3 | Open field arena test (OFT)

The motor function study was performed as per the procedure recommended previously with a minor amendment.[35] The locomotor activity (motor dysfunction) of adult zebrafish was evaluated by OFT and the apparatus is a 5-L rectangular tank (L × W × H:21 × 21 × 15 cm) filled with 3-L aquarium water. As per the usual behavior, zebrafish swim to and fro along the length of the open field arena and thus this common observation was considered to ascertain the locomotor movement of zebrafish. The OFT was virtually divided into nine equal zones (the length of each zone was 7 cm) by three vertical and horizontal lines. The locomotor activity was recorded for 5 min by the ANYmaze system by analyzing the time taken to cross all zones, total distance traveled, and mean velocity in OFT ($n$ = 18/group).

### 2.4.4 | Oxidative stress parameters

After the conclusion of BPA exposure and neurobehavioral study, zebrafish were sacrificed and brains were dissected out at 4°C ($n$ = 18/group). For the biochemical assay, a cluster of six whole brains was used for each experimental sample. Then, the brains were softly ground in ice-cold radioimmunoprecipitation buffer (RIPA) in a glass homogenizer, and then the mixture was incubated for 25 min at 4°C to collect the supernatant for further use in biochemical assays.[36]

### 2.4.5 | Protein carbonylation study to evaluate protein oxidation rate

Protein carbonylation is the quantification of protein-bound carbonyls following protein oxidation.[37] Briefly, 10% of homogenate of zebrafish brain in RIPA buffer was centrifuged at 12,000 rpm/20 min and then the supernatant was separated. Subsequently, 0.5 ml of the collected supernatant was treated with 0.5 ml 10 mM DNPH (2,4-dinitrophenylhydrazine) in 2 M HCl for 1 h at room temperature (RT) and was subjected to vortex at 15 min intervals. Later, 0.5 ml of 20% TCA (trichloroacetic acid) were mixed and centrifuged at 11,000$g$ at 4°C for 10 min. The pellet obtained was washed three times with 1 ml of ethanol–ethyl acetate (in the ratio 1:1) to eliminate the unreactive reagent. Subsequently, the pellet protein was dissolved in 400 µl of 6 M guanidine and then centrifuged at 10,000 g at 4°C for 10 min to remove any residues; finally, the carbonyls were measured at 366 nm with the help of a spectrophotometer. A blank control was taken by incubation with 2 M HCl without DNPH. The carbonyl content was measured using the molar extinction coefficient of aliphatic hydrazone and the results were expressed as nMole carbonyl per mg of protein.

### 2.4.6 | Lipid peroxidation as a measure of thiobarbituric acid reactive substance (TBARS)

The formation of TBARS is considered the measure of lipid peroxidation.[34] Briefly, 100 µl of the aliquoted brain supernatant was mixed with 3.8 ml of thiobarbituric acid reagent and incubated at 95°C for approximately 60 min in a water bath, and then, it was centrifuged at 10,000$g$ for 10 min. At that point, a pink chromogen was produced; it was analyzed at 532 nm with the help of a spectrophotometer. This experimental test evaluates malondialdehyde (MDA), which is a breakdown fraction of an endoperoxide of unsaturated fatty acids resultant from lipid component oxidation.[38] The MDA and thiobarbituric acid (TBA) underwent condensation reaction to form a pink color pigment (pink chromogen) and spectrophotometric analysis of pink chromogen delivered an outcome towards the rate of lipid peroxidation. In conclusion, the TBARS concentration was determined from its extinction coefficient and the results were expressed as nMole TBARS formed/mg of protein.

### 2.4.7 | Reduced glutathione (GSH)

The level of reduced GSH in the tissue can be considered the direct measure of the low level of cytosolic oxidative stress. In this study, the GSH level of tissue homogenate of zebrafish brain was determined according to the protocol described earlier.[39] In a nutshell, approximately 200 µl of brain supernatant was added up to the identical capacity of phosphoric acid and subsequently, the mixture solution was subjected to centrifugation at 4000 g at 4°C for 15 min. Then the supernatants were collected and used for the measurement of GSH by 30 min incubation at RT with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB). Finally, the mixture solution was subjected to spectrophotometric analysis taken at 412 nm and the amount of GSH was interpreted using a standard curve and expressed in µmole/g of tissue.

### 2.4.8 | Glutathione reductase (GR) assay

The GR activity assay in zebrafish brain was quantified according to the protocol defined earlier.[40] The degree of change of GSSG to GSH was calculated by studying the NADPH oxidation spectrophotometrically at 340 nm. The glutathione reductase activity was determined using the molar extinction coefficient of NADPH and represented as nMole NADPH oxidized/min/mg protein.

### 2.4.9 | Neuromorphological study by Cresyl violet staining (CVS)

To study neuronal pyknosis, CVS was conducted according to a previously reported protocol.[41] Shortly after the completion of requisite exposure and behavioral tests, the complete zebrafish brain was removed at 4°C individually and then treated with 30% sucrose solution ($n$ = 6/group). Cryosectioning was performed with the help of a cryostat (Leica Biosystems), 10 µm thick sections were collected in ice-cold phosphate-buffered saline (PBS) and CVS was performed. The mounted slides with the stained sections were analyzed with the help of a microscope (Olympus, CX31) and the respective images of the sections were captured with necessary magnification for further analysis. The neurons displaying the staining pattern of pyknosis were enumerated by stereo Investigator software (MBF Bioscience). The results thus obtained were denoted as a percent of control (control values considered as 100%).

### 2.4.10 | Chromatin condensation study by Hoechst staining

The incidence of chromatin condensation, which is a marker of apoptotic cell death, was examined by Hoechst staining.[42] In the present study, chromatin condensation by Hoechst 33342 dye was carried out separately for all experimental groups ($n$ = 6/group). The zebrafish brain sections obtained after cryosectioning were permeabilized with 0.1% triton and subjected to staining with Hoechst 33342 (1.5 µg/ml) for 5–7 min in the dark at room temperature. Then, the cryosection was subjected to gentle washing with 0.1 M PBS and finally mounted through glycerol. The stained brain sections were analyzed by using a blue filter (DAPI [4′,6-diamidino-2-phenylindole]) in a microscope (Olympus-CX31) and the chromatin compression with nuclear injury was recorded by scoring the quantity of Hoechst positive cells in four arbitrary non-overlying arenas of 0.1 $mm^2$ producing positive blue fluorescence at ×40 magnification. The mean results thus found for each experimental group were transformed to a percentage by considering control results as 100%.

### 2.4.11 | Immunohistochemistry for cleaved (activated) caspase-3 expression

The immunohistochemistry was performed as per the protocol described previously with minor modification.[43,44] For immunolabeling of diencephalic and telencephalic neurons in zebrafish brain, the requisite brain sections were first subjected to an antigen recovery step by incubation in 10 mM sodium citrate buffer at 95°C in a water-bath for 15 min ($n$ = 6/group). Then the slides were cool down at room temperature and washed twice with 1× PBS. Subsequently, the sections were permeabilized by incubation in 1× PBS containing 0.3% Triton-X-100 buffer for 30 min. Sections were washed three times with wash buffer (1× PBS holding 0.1% Triton-X-100) for 10 min each and later the sections were incubated in 5% normal Goat serum in 1× PBS containing 0.1% Triton-x 100 at RT for 60 min followed by overnight incubation at 4°C with cleaved (activated) caspase-3 primary antibody (1:200).[45] The excess primary antibody was cast off from sections by washing three times with wash buffer for 10 min each at RT. Then the sections were incubated in secondary antibody (1:500, goat anti-Rabbit IgG [H+L]; Alexa Flour 568) for 2 h at RT. Sections were washed three times with wash buffer for 10 min each. Then the sections were counterstained with DAPI and washed three times in distilled $H_2O$ for 5 min each. Sectioned were shielded by coverslip after incorporation of anti-fade reagent.

### 2.4.12 | Immunoblotting for targeted protein expression

A protein expression study by western blot was carried out.[41,43] The whole brains were surgically dissected out and homogenized in lysis buffer (20 mM Tris, pH 8.0; 1 mM, EDTA; 0.5 mM, EGTA; 0.1% sodium deoxycholate; 150 mM NaCl; 1% IGEPAL; 10% glycerol) supplemented with protease inhibitor cocktail (Roche) and 1 mM PMSF. Protein lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and after transfer into nitrocellulose membrane (NCM), the membranes were blocked with 5% skimmed milk for 1 h and then washed away with 0.01 M PBS, PBST, and PBS. After that, NCMs were subjected to incubation in desired

primary antibodies for 8–10 h at 4°C, then washed three times (PBS/PBST/PBS). Horseradish peroxidase-conjugated secondary antibody was used for the final step in immunoblotting. At last, the NCMs were again subjected to a similar final step of washing and the signal was captured by using a chemiluminescent peroxidase substrate kit. ImageJ software was used for densitometric analysis of capture signal blot and then the values were expressed as a percent of control.

#### 2.4.13 | Statistical analysis

The statistical results of all experimental groups and parameters were represented as mean ± SEM (standard error of the mean). The statistical findings were compared using a one-way analysis of variance and posthoc analysis by Newman–Keuls posthoc test. Altogether, the significance level was considered as $p < 0.05$.

## 3 | RESULTS

### 3.1 | BPA altered the scototaxis behavior of zebrafish

The representative track plot of LDPT of all experimental groups showed that there was a substantial transformation in scototaxis behavior of zebrafish following chronic exposure to BPA (Figure 1A). In LDPT, the light zone time spent [$F_{3,68} = 16.02$, $p < 0.05$] and latency to enter dark zone [$F_{3,68} = 10.4$, $p < 0.05$] were significantly higher in BPA-treated groups (BPA1.0 and BPA4.0) in comparison with naïve and control (Figure 1C,D). BPA treated groups also showed a significant increase in the number of transitions/entries into the light zone in LDPT when compared with the control group [$F_{3,68} = 20.06$, $p < 0.05$] (Figure 1B). No substantial alteration in behavioral parameters was recorded between the naïve and control groups. A higher concentration of BPA (BPA4.0) showed significant alteration in the scototaxis behavior of zebrafish as compared to BPA1.0 [$F_{3,68} = 16.02$, $p < 0.05$] (Figure 1B–D).

### 3.2 | BPA transformed the explorative and locomotory behavior of zebrafish

The representative track plot of NTDT of all investigational groups displayed significant alteration in explorative behavior of zebrafish following exposure to BPA (Figure 2A). In NTDT, the total distance traveled [$F_{3,68} = 5.411$, $p < 0.05$] and mean velocity [$F_{3,68} = 14.24$, $p < 0.05$] in the diving tank following chronic BPA exposure was significantly decreased in comparison to naïve and control (Figure 2B,C). The top zone transitions [$F_{3,68} = 12.85$, $p < 0.05$] were also significantly increased in BPA groups when compared with naïve and control (Figure 2D). Chronic waterborne exposure to a higher concentration of BPA (BPA4.0) significantly altered the total distance traveled and mean velocity in NTDT when compared with the BPA1.0 group. These findings showed that chronic waterborne exposure to BPA induces locomotor dysfunction in zebrafish, possibly leading to Parkinsonism-like phenotypes.

### 3.3 | Chronic waterborne exposure to BPA induces locomotor dysfunction in zebrafish

The locomotor dysfunction study following exposure to BPA was further corroborated by the OFT and the representative track plots depicted considerable alteration in the locomotory activity

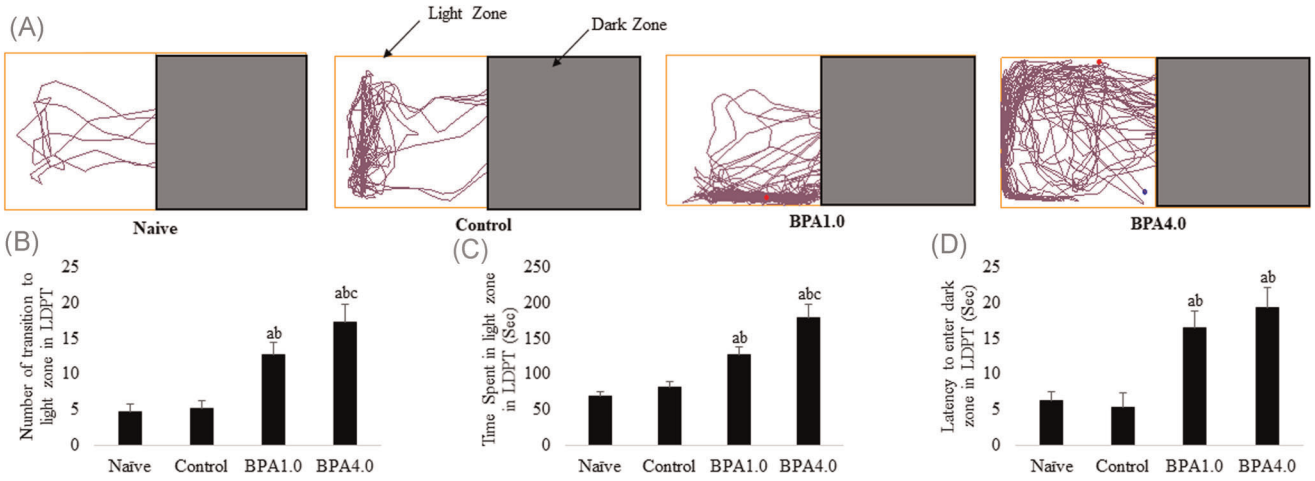


**FIGURE 1** Light/dark preference test (LDPT). Representative track sheets of LDPT (A). Graphs showing significant variation in the number of transitions to light zone (B), time spent in the light zone (C), and latency to enter dark zone (D) for a 5-min session in LDPT following chronic waterborne BPA exposure. Values are expressed as mean ± SEM. "a" denotes $p < 0.05$ when compared to a naive group, "b" denotes $p < 0.05$ when compared to the control group, and "c" denotes $p < 0.05$ when compared to the BPA1.0 group

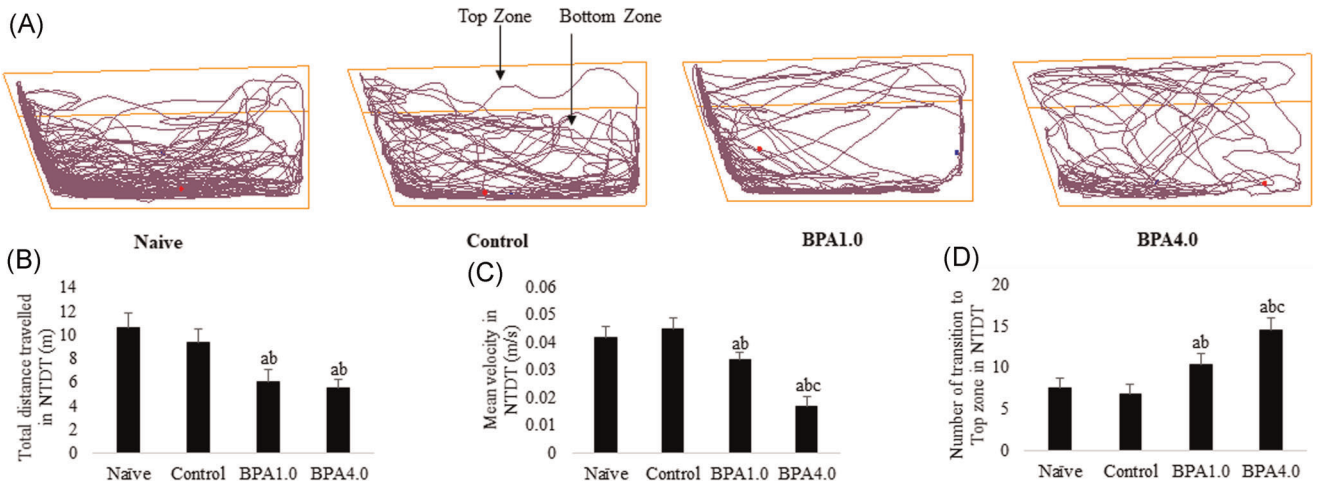


**FIGURE 2** Novel tank diving test (NTDT). Representative track sheets of NTDT (A). Graphs showing alteration in the total distance traveled (B), mean velocity (C), and number of transitions to top zone (D) in novel tank diving test following waterborne exposure to BPA. Values are expressed as mean ± SEM. "a" denotes $p < 0.05$ when compared to the naive group, "b" denotes $p < 0.05$ when compared to the control group and "c" denotes $p < 0.05$ when compared to the BPA1.0 group

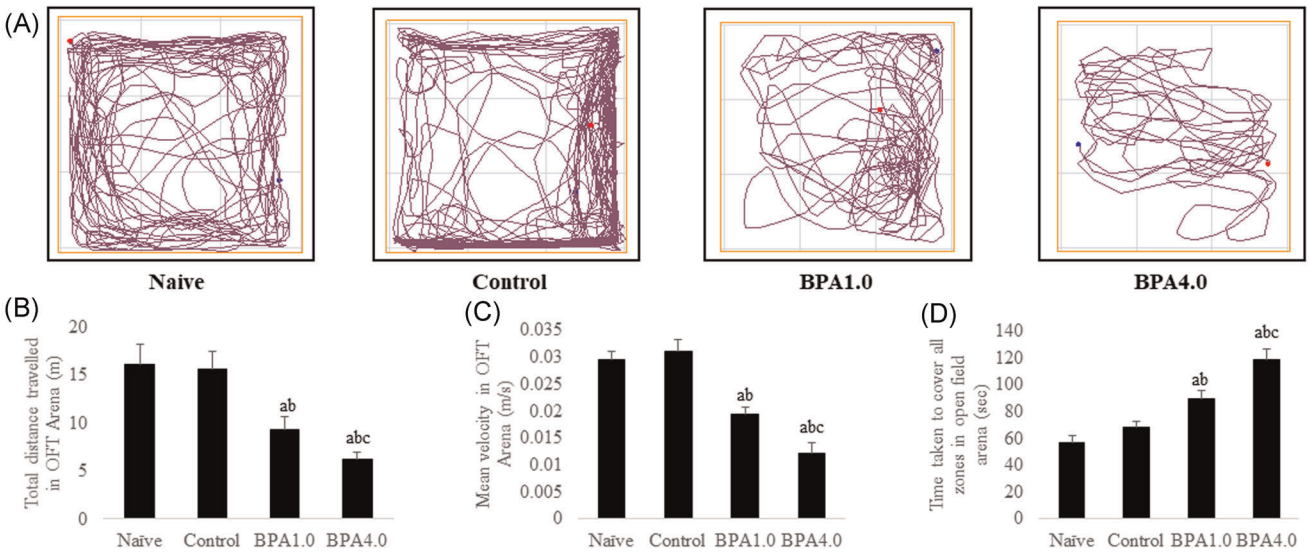


**FIGURE 3** Open field arena test (OFT). Representative track plots of OFT (A). Graphs displaying variations in the total distance traveled (B), mean velocity (C), and time taken to cover all zones (D) in OFT following waterborne exposure to BPA. Values are expressed as mean ± SEM. "a" denotes $p < 0.05$ when compared to the naive group, "b" denotes $p < 0.05$ when compared to the control group and "c" denotes $p < 0.05$ when compared to the BPA1.0 group. BPA, bisphenol A

of zebrafish (Figure 3A). The basic observation in OFT implied a significant reduction in total distance traveled [$F_{3,68} = 12.47$, $p < 0.05$] and mean velocity [$F_{3,68} = 23.27$, $p < 0.05$] in BPA treated groups as compared to naïve and control (Figure 3B,C). Our alternate observation with respect to the average time taken to cross all zones in OFT [$F_{3,68} = 19.77$, $p < 0.05$] was significantly reduced in the BPA4.0 group as compared to BPA1.0, naïve and control groups (Figure 3D). The general observation of the study inferred that chronic exposure to BPA significantly impaired motor function and thus induces PD-like phenotypes in zebrafish.

### 3.4 | BPA exposure induces oxidative stress in the brain

Exposure to BPA for 56 days significantly decrease the reduced GSH level [$F_{3,68} = 20.58$, $p < 0.05$] and GR activity [$F_{3,68} = 66.19$, $p < 0.05$] in zebrafish brain as compared to naive and control (Figure 4A,B). Additionally, lipid peroxidation [$F_{3,68} = 55.26$, $p < 0.05$] and protein carbonylation [$F_{3,68} = 85.92$, $p < 0.05$] were found to be considerably augmented in BPA groups (BPA1.0 and BPA4.0) when compared with naïve and control (Figure 4C,D). Higher concentrations of BPA adversely altered the level of antioxidant, protein carbonylation, and

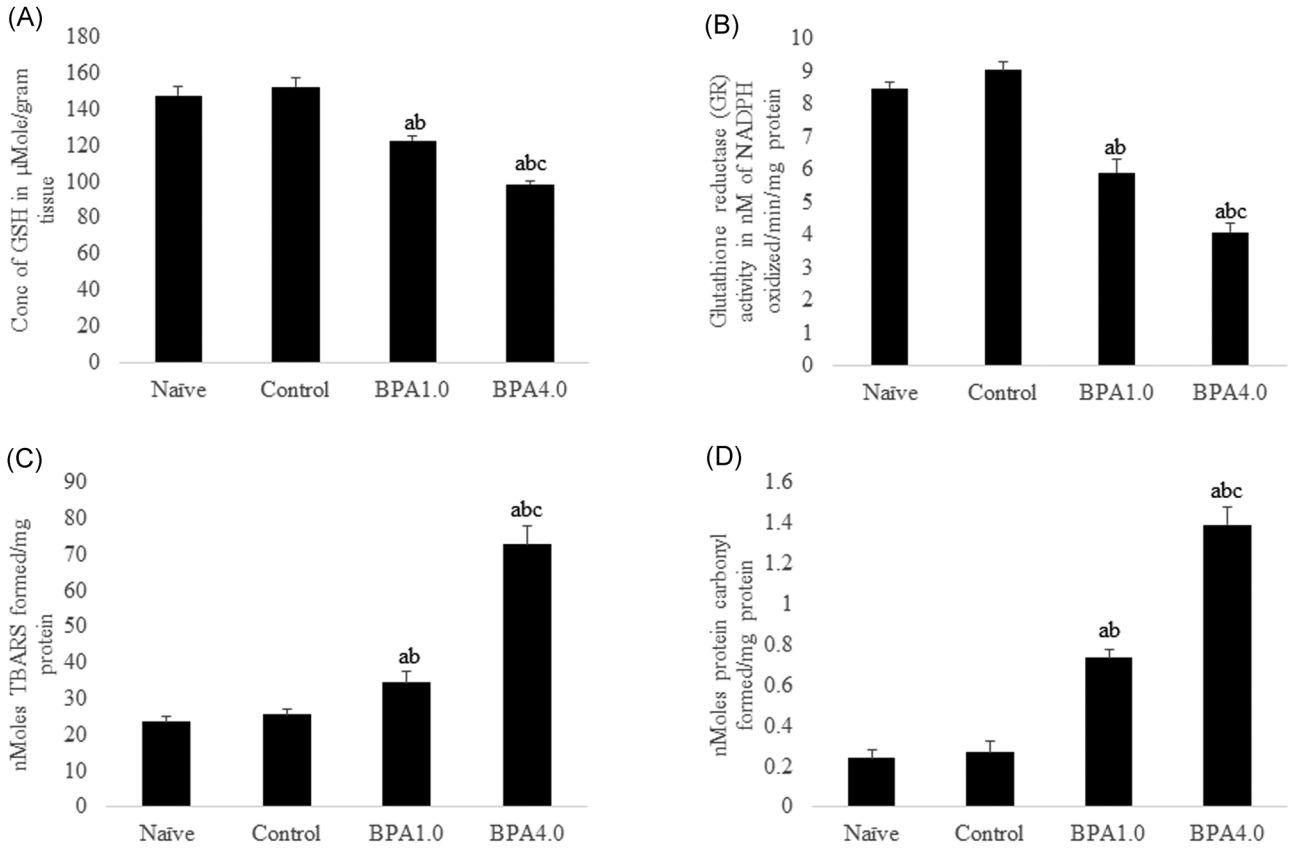


**FIGURE 4** Oxidative stress parameter assay. Graphs representing changes in the concentration of GSH (µMole/gram of tissue) (A) and GR (nM of NADPH oxidized/min/mg protein) (B) in the whole brain of zebrafish following chronic waterborne BPA exposure. Graphs representing changes in nMoles TBARS formed/mg protein as a measure of lipid peroxidation (C) and nMoles protein carbonyl formed/mg protein as a measure of protein carbonylation (D) in the whole brain of zebrafish following chronic waterborne BPA exposure. Values are expressed as mean ± SEM. "a" denotes $p < 0.05$ when compared to the naive group, "b" denotes $p < 0.05$ when compared to the control group, and "c" denotes $p < 0.05$ when compared to the BPA1.0 group. BPA, bisphenol A; GSH, reduced glutathione; GR, glutathione reductase; TBARS, thiobarbituric acid reactive substance

lipid peroxidation in the zebrafish brain. The findings of the present study show that BPA induces oxidative stress leading to a change in the antioxidant level (GSH) in the zebrafish brain as compared to naïve and control.

### 3.5 | Augmented neuronal pyknosis in zebrafish brain following chronic BPA exposure

Neuromorphological observation of the diencephalon [$F_{3,20} = 33.05$, $p < 0.05$] and telencephalon [$F_{3,20} = 27.38$, $p < 0.05$] regions of zebrafish brain by cresyl violet staining show a substantial upsurge in pyknotic neuronal counts in BPA treated groups in comparison to naïve and control (Figure 5A,B). Furthermore, increasing the concentration of waterborne BPA (BPA4.0) exposure significantly augmented the pyknotic cell count as compared to the BPA1.0 group. No substantial alteration in the pyknotic neuronal count was recorded in zebrafish of naive and control groups (Figure 5A,B). These observations provide the basic ideas that the sensitive brain regions of zebrafish which regulate the locomotor activity through the availability of dopaminergic neurons, might be affected by chronic waterborne exposure to BPA.

### 3.6 | Chronic waterborne exposure to BPA leads to chromatin condensation

Chromatin condensation in the diencephalic and telencephalic regions was counted by Hoechst 33342 staining following waterborne exposure to different concentrations of BPA (Figure 6A,B). Percentage of Hoechst-positive neurons in diencephalon [$F_{3,20} = 12.38$, $p < 0.05$] and telencephalon [$F_{3,20} = 34.24$, $p < 0.05$] of zebrafish brain gradually increased in BPA1.0 and BPA4.0 groups (Figure 6C,D) in comparison to naïve and control.

### 3.7 | BPA exposure significantly augment the expression of cleaved caspase-3 in brain regions of zebrafish

To validate the involvement of caspase-mediated apoptotic cell death in BPA-induced neurodegeneration in the diencephalic and telencephalic regions of zebrafish brain, we performed an immunohistochemical study for determining the change in the expression pattern of activated (cleaved) caspase-3 (Figure 7A,B). The results demonstrate that there was

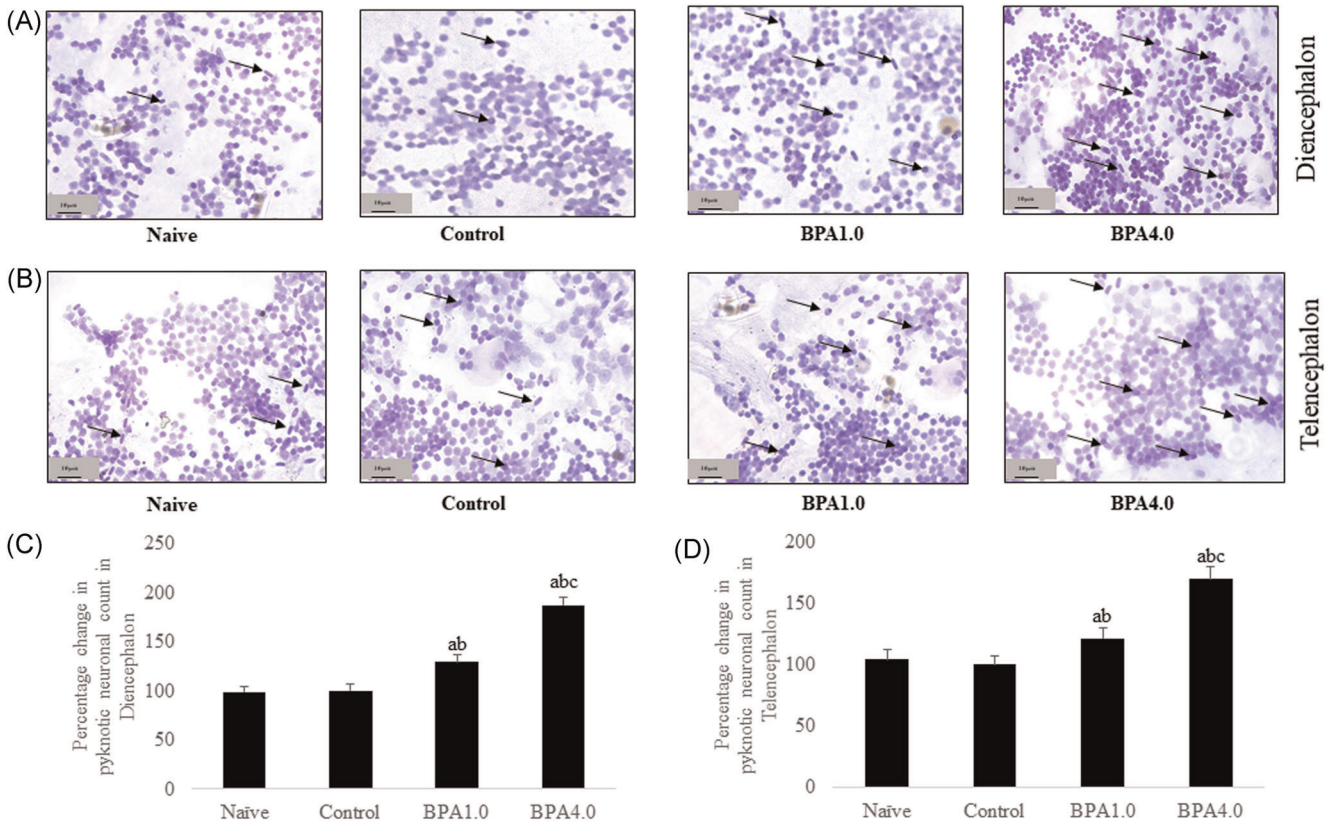


**FIGURE 5** Neuromorphological study of zebrafish brain by cresyl violet staining. (i) Representative image of whole-brain cross-sectional view following cresyl violet staining of diencephalon (A) and telencephalon (B) of the zebrafish brain. Arrowheads depict pyknotic cells. Magnification and scale bar: ×40 corresponds to 10 μm of different experimental groups. Graph showing percentage change in pyknotic neuronal counts in the diencephalon (C) and telencephalon (D) of zebrafish brain following chronic waterborne exposure to BPA. Values are expressed as mean ± SEM. "a" denotes $p < 0.05$ when compared to the naive group, "b" denotes $p < 0.05$ when compared to the control group, and "c" denotes $p < 0.05$ when compared to the BPA1.0 group

a substantial upsurge in the expression of cleaved caspase-3 in diencephalon [$F_{3,20} = 18.53$, $p < 0.05$] and telencephalon [$F_{3,20} = 16.17$, $p < 0.05$] of zebrafish brain following chronic waterborne exposure to BPA when compared with naïve and control (Figure 7C,D).

### 3.8 | Alterations in the expression pattern of marker proteins of Parkinson's disease (PD) following chronic BPA exposure

Whole-brain neuronal nuclei (NeuN) expression by immunoblotting showed to be downregulated following chronic waterborne exposure to BPA [$F_{3,68} = 9.52$, $p < 0.05$] (Figure 8B). Dopaminergic neuronal loss is considered an important factor for the development of PD like phenotypes. Our findings showed that tyrosine hydroxylase (TH: a marker of dopaminergic neurons) was suggestively downregulated in BPA1.0 and BPA4.0 groups when compared with the control [$F_{3,68} = 11.95$, $p < 0.05$] (Figure 8E). Additionally, nurr1 (nuclear receptor-related 1: an activator of TH expression was expressively downregulated in BPA treated groups when compared to naïve and control [$F_{3,68} = 12.56$, $p < 0.05$] (Figure 8D). Alpha-synuclein (α-Syn) [$F_{3,68} = 12.69$, $p < 0.05$] and LRRK2 [$F_{3,68} = 9.912$, $p < 0.05$] expression of whole-brain was found to be upregulated in the presence of BPA (Figure 8C,F). Our result also demonstrates that there was a significant augmentation in cleaved caspase-3 expression in BPA groups in comparison with naïve and control [$F_{3,68} = 21.70$, $p < 0.05$] (Figure 8G).

## 4 | DISCUSSION

Due to anthropogenic activities, the load of neurotoxicants is increasing in inhabiting soil, air, and water which poses a plausible risk in the form of the development of serious neurological diseases. BPA is typically anthropogenic in origin and ubiquitous in modern-day life. The mode of human exposure to BPA involves the digestive system, respiration, and dermal contact.[46] Earlier reports have shown that BPA exposure causes an increase in anxiety, oxidative stress, carcinogenicity, impairs spatial memory, neuroinflammation, and neurobehavioral alterations.[3–7]

The current experimental paradigm was set to elucidate the potent role of BPA towards the progression of PD-like features in

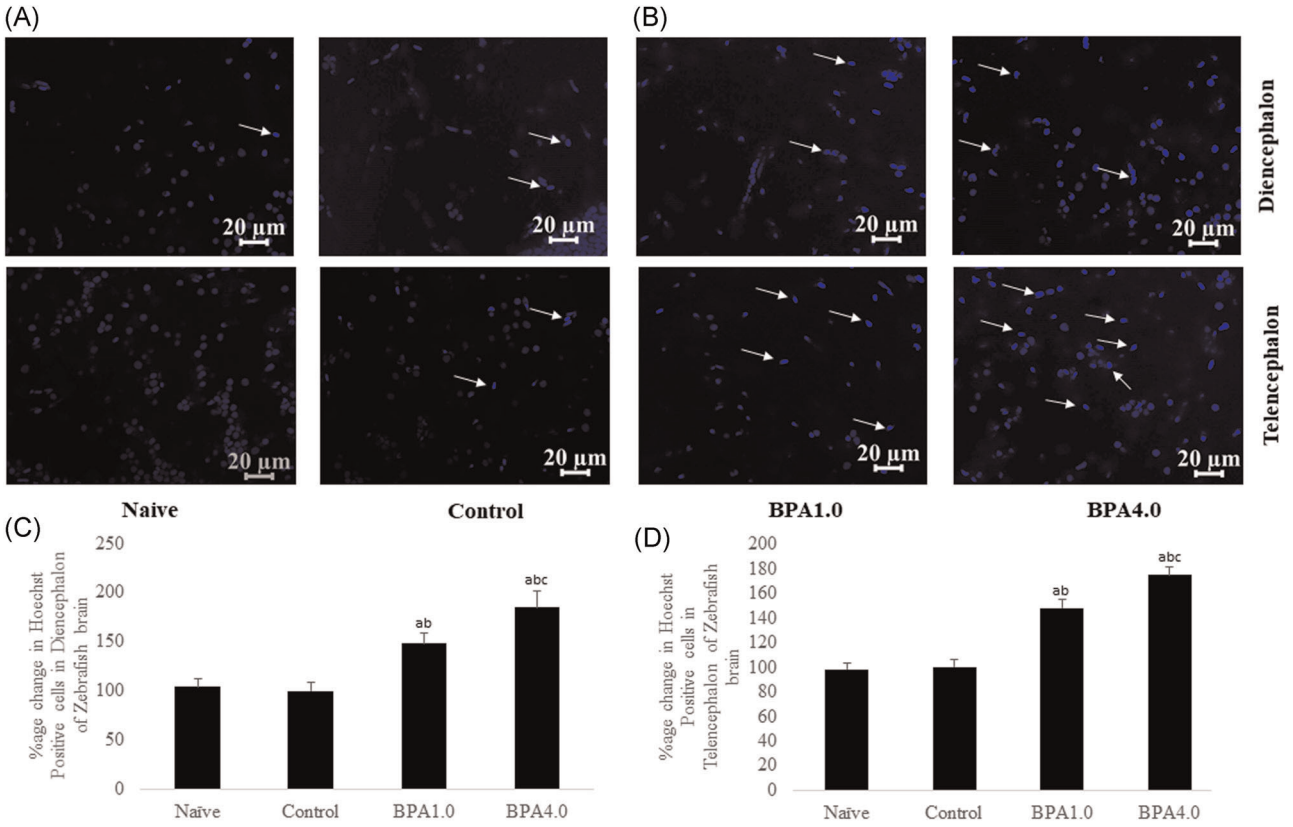


**FIGURE 6** Chromatin condensation study by Hoechst staining. (i) Representative image of requisite zebrafish brain regions after Hoechst 33342 staining of diencephalon (A) and telencephalon (B). Arrowheads depict Hoechst-positive neurons. Magnification and scale bar: ×40 corresponds to 20 µm of different experimental groups. Graph showing percentage changes in Hoechst-positive neurons in the diencephalon (C) and telencephalon (D) of zebrafish brain following chronic waterborne exposure to BPA. Values are expressed as mean ± SEM. "a" denotes $p < 0.05$ when compared to the naive group, "b" denotes $p < 0.05$ when compared to the control group, and "c" denotes $p < 0.05$ when compared to the BPA1.0 group. BPA, bisphenol A

zebrafish. Two different concentrations of BPA were selected (1.0 mg/L [4.38 µM] and 4.0 mg/L [17.52 µM]) for chronic waterborne exposure which were considerably higher than ecologically related levels to create a condition to predict the substantial costs of swelling pollution accumulation on aquatic animals. In the present study, our findings demonstrated that chronic exposure to BPA for 56 days potentiates the development of locomotor dysfunction, oxidative stress, transformed expression of PD-relevant markers and augmented neurodegeneration in the sensitive brain region of zebrafish. Furthermore, the literature also depicted that BPA exposure significantly downregulates the expression of TH following 7 weeks of BPA exposure and is in line with our observations with 56 days of BPA exposure.[47] Similarly, another study also reported performed a long-duration BPA exposure (6 months) to study its impact on locomotor activity and other behavioral parameters.[48] The current results displayed that chronic BPA exposure suggestively transformed the usual behavioral responses, therefore endorsing an anxiety-like characteristic with an augmented visit to the light zone in LDPT. Additionally, NTDT also showed a decreased time spent in the top zone as compared to the bottom zone following BPA exposure, which is unorthodoxy from the usual behavior of zebrafish.[49] To validate the BPA-induced locomotor dysfunction, we performed an alternative OPT and the basic observation shows that there was a substantial alteration in total distance traveled and mean velocity in OFT in BPA treated group as compared to naïve and control. Furthermore, the time taken to cross all assigned zones in OFT was substantially increased in BPA exposed groups. These findings advocate the potent role of BPA in inducing motor dysfunction in zebrafish leading to development of PD-like features. These basic conclusions advocate that BPA exposure for a considerable time may aggravate the chances towards development of locomotor impairment matching to PD-like features.

BPA has a straightforward role in shifting the brain's antioxidant composition and antioxidant enzyme activity. The observation of the current study correspondingly shows a substantial upsurge in protein carbonylation and lipid peroxidation in the brain of BPA-treated groups, which could be through increased oxidative stress. The main objective of the present study on zebrafish is to analyze the adverse effect of increasing BPA load in water bodies and its association with the development of Parkinsonism-like phenotypes. Furthermore, oxidative stress might be triggered by diverse reasons that decline the level of antioxidants, such as reduced GSH with succeeding

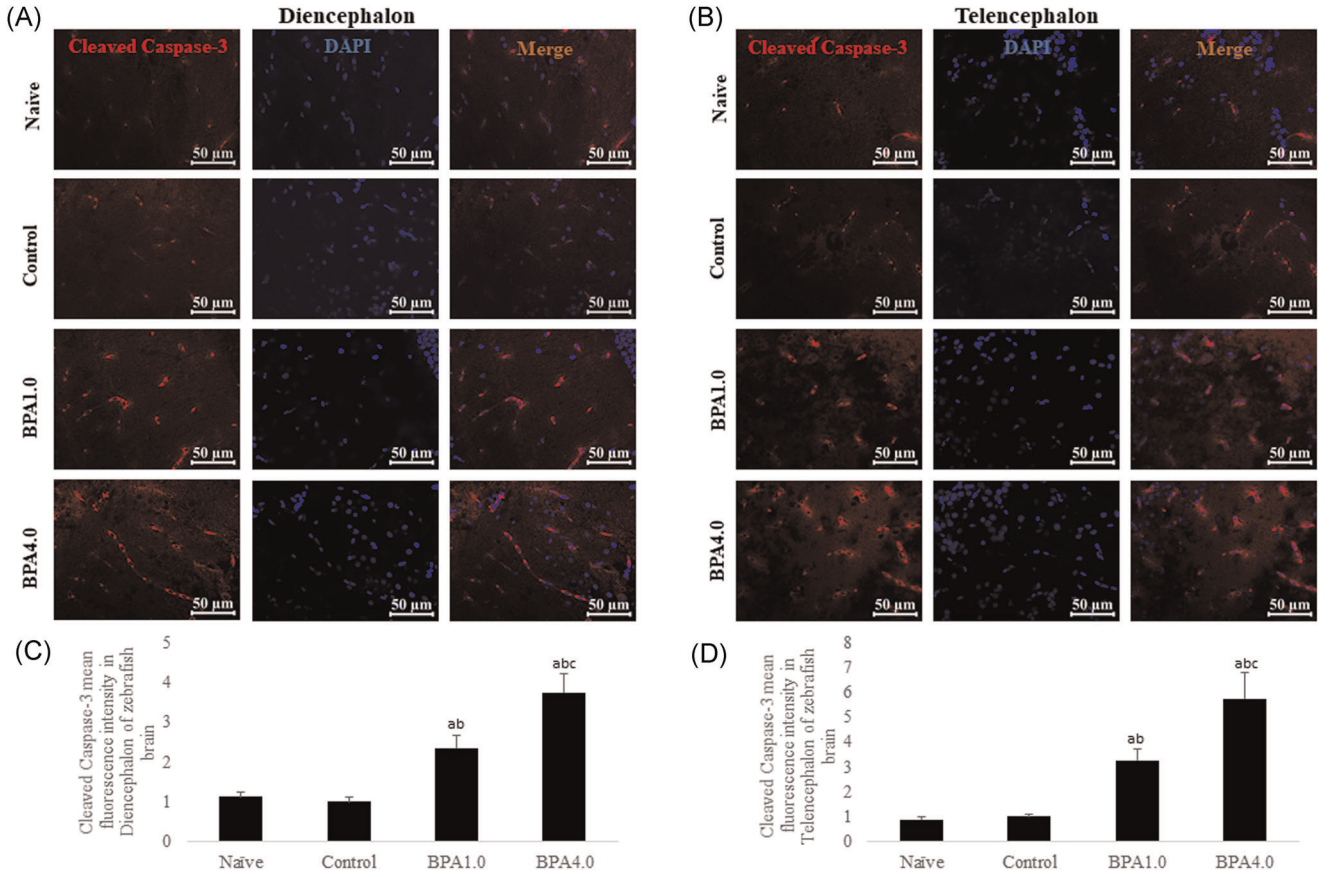


**FIGURE 7** Immunohistochemistry for activated caspase-3 expression in zebrafish brain. (i) Representative image depicting expression of activated (cleaved) caspase-3 following immunohistochemistry of diencephalon (A) and telencephalon (B) of zebrafish brain. Red channel depicts cytoplasmic cleaved caspase-3 and blue channel (DAPI) for nucleus staining. Magnification and scale bar: ×40 corresponds to 50 µm of different experimental groups. Graph showing changes mean fluorescence intensity of activated caspase-3 expression in the diencephalon (C) and telencephalon (D) of zebrafish brain following chronic waterborne exposure to BPA. Values are expressed as mean ± SEM. "a" denotes $p < 0.05$ when compared to the naive group, "b" denotes $p < 0.05$ when compared to the control group, and "c" denotes $p < 0.05$ when compared to the BPA1.0 group. BPA, bisphenol A; DAPI, 4′,6-diamidino-2-phenylindole

upsurge in reactive oxygen species (ROS) production. Oxidative stress also plays a vital part in the pathophysiology of various neurological conditions.[50] The level of GSH in the brain can be augmented securely by means of diverse treatment approaches and its upsurge will be having neuroprotective benefits against serious neurological disorders.[51] In addition, chronic waterborne exposure to BPA induces a reduction in the GSH level and glutathione reductase activity in the whole-brain lysate of zebrafish. As cellular GSH is available at a substantial level, it guards cells against free radical generation.[52] Our findings showed that oxidative stress-persuaded reduction of antioxidant enzymes undertakes by the augmented generation of ROS during chronic BPA exposure.

The neurotoxic ability of BPA has also been talked about in animal models.[53] As BPA has a possible role in shifting neurobehavioral response and rising oxidative stress displaying its potential negative role, we postulated that chronic waterborne exposure to BPA may develop PD-like features in zebrafish. Hence, we performed the neuromorphological study of the diencephalic and telencephalic regions of the brain after chronic BPA treatment and the outcome of the study presented a substantial upsurge in pyknotic neuronal counts in these regions compared to those in naïve and control. A higher concentration of water-borne BPA exposure leads to augmented neuronal pyknosis leading to neurodegeneration. Chromatin condensation study was also performed by Hoechst 33342 staining to validate that the BPA induced neurodegeneration in diencephalon and telencephalon was an outcome of probable apoptotic neuronal death in zebrafish. Our findings show that there was a substantial increase in Hoechst-positive neurons in the diencephalon and telencephalon of the zebrafish brain following exposure to BPA. To authenticate the contribution of caspase facilitated apoptotic cell death in BPA encouraged neurodegeneration in diencephalon and telencephalon, we performed immunostaining for shaping the alteration in activated (cleaved) caspase-3 expression. Our finding strongly demonstrated a substantial upsurge in activated caspase-3 expression in the diencephalic and telencephalic of the zebrafish brain following chronic exposure to BPA.

A marker-based study to address the neurodegenerative potential of anthropogenic toxicants like BPA has been carried out in the

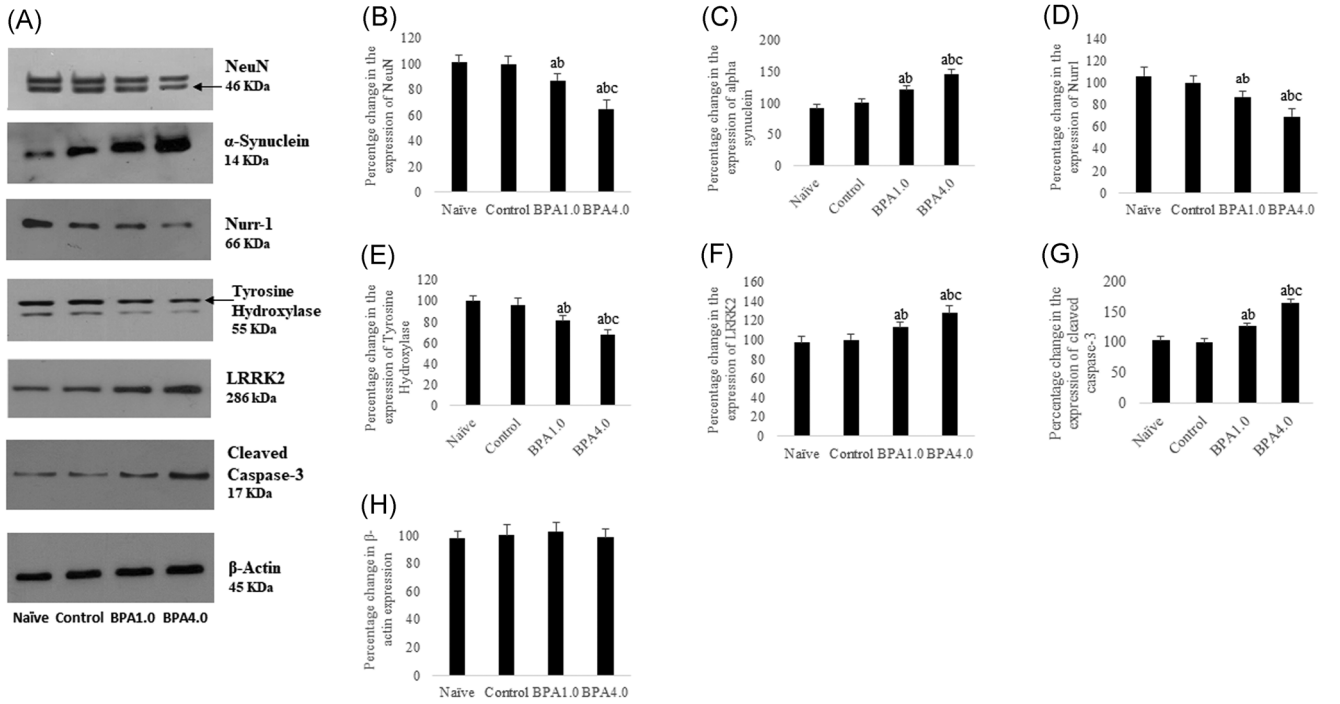


**FIGURE 8** Protein expression study by western blot analysis. Representative immunoblots of targeted proteins (A). Graphs showing changes in expression of NeuN (B), α-synuclein (C), Nurr1 (D), tyrosine hydroxylase (E), LRRK2 (F), cleaved caspase-3 (G), and β-actin (H). Lanes from left to right denote Naïve, Control, BPA1.0, and BPA4.0 (A). Corresponding graphs denote mean ± SEM. Values are expressed as mean ± SEM. "a" denotes $p < 0.05$ when compared to the naive group, "b" denotes $p < 0.05$ when compared to the control group, and "c" denotes $p < 0.05$ when compared to the BPA1.0 group. β-Actin was used as the loading control

present study. TH is an enzyme playing a crucial role in the synthesis of L-DOPA. Consequently, PD can be termed a TH-deficit disorder pronounced towards a decrease in the DA level. A previous report firmly suggested that the damage or loss of TH-positive neurons resultant from the decrease of the DA in PD is very common.[54] Further reports proposed that Nurr1 plays a critical role in the maintenance and survival of the dopaminergic system of the brain.[55,56] The Nurr1 protein is supposed to play a pivotal role in the development of the dopamine phenotype in the midbrain.[57] Earlier reports also advocated that Nurr1 induces TH expression, which eventually differentiates into dopaminergic neurons.[58] Therefore, downregulation of Nurr1 can lead to loss of dopaminergic neurons, a vital symbol of PD symptoms. On the contrary, α-Syn, is connected neuropathologically and genetically with Parkinson's.[59] Reports also advocated that α-Syn limits the function of TH by hindering the rate of TH phosphorylation.[60] Progressively, α-Syn aggregates to produce unsolvable fibrils in pathological conditions characterized by Lewy bodies (LBs), a trademark of Parkinson's disease. [59,61]

To elucidate the likely role of BPA in persuading Parkinsonism-like phenotypes, we have studied the expression pattern of PD-specific marker proteins. The results of the current study validate that the locomotor impairment resulting from chronic waterborne exposure to BPA in zebrafish is rightly interrelated with reduction in TH and Nurr1 in the brain leading to lessening in DA level. Additionally, NeuN was expressively downregulated in BPA exposed group. The level of α-Syn was raised in the zebrafish brain following exposure to BPA. As a marker of apoptotic cell death, the expression of cleaved caspase-3 was considerably augmented in BPA exposed groups. A higher concentration of BPA significantly transformed the alteration in marker proteins of PD in the brain. Hence, the current study advocates the ground-breaking finding revealing the straightforward connotation of BPA and neurodegenerative disease in zebrafish. Tactical guidelines on the disposal of environmental genotoxicants[62,63] like BPA into/nearby water bodies could lessen the impact on aquatic fauna and human health.

## 5 | CONCLUSION

The current findings established the likely protagonist role of BPA-persuaded neurodegeneration leading to the progress of PD-like features in zebrafish. Additionally, the results of the study also gave conclusive evidence that BPA is well competent in inducing locomotor impairment, oxidative damage leading to neurodegeneration, and development of Parkinsonism-like phenotypes. Nevertheless, the gross observation of the current study also demonstrated the adversative effect of chronic BPA exposure on neurobehavioral, biochemical, and neuromorphology in zebrafish. Future studies leading in the direction of understanding the downstream signaling cascades of BPA-induced neurodegeneration might deliver novel understanding into the development of prophylactic strategies to avert the development of neurodegenerative diseases.

## ACKNOWLEDGMENTS

The authors acknowledge the Centre for Biotechnology, Siksha 'O' Anusandhan (Deemed to be University) for providing the infrastructure facility and support. Dr. Das is the recipient of UGC start-up and ICMR extramural (Multi-Institutional) grants. The authors also acknowledge the contribution of Dr. Ritendra Mishra, Assistant managing editor, Crimson interactive Pvt. Ltd., Mumbai, India who helped in the copy editing and proofreading of the manuscript.

## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

*Designed the experiments, secure the funding, and wrote the main theme of the manuscript*: Saroj K. Das. *Performed experiments*: Pradyumna K. Sahoo and Sai Aparna. *Gave critical inputs, contributed to the literature review*: Pradeep K. Naik and Shashi B. Singh. All authors contributed to the drafting of the manuscript, reviewed the manuscript, and have given their consent to the final version of the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

*Pradyumna K. Sahoo* http://orcid.org/0000-0003-1505-2893
*Saroj K. Das* http://orcid.org/0000-0002-5717-5333

## REFERENCES

[1] R. E. Chapin, J. Adams, K. Boekelheide, L. E. Gray Jr., S. W. Hayward, P. S. Lees, B. S. McIntyre, K. M. Portier, T. M. Schnorr, S. G. Selevan, J. G. Vandenbergh, S. R. Woskie, *Birth. Defects. Res. B. Dev. Reprod. Toxicol.* **2008**, *83*(3), 157. https://doi.org/10.1002/bdrb.20147

[2] H. Fukazawa, K. Hoshino, T. Shiozawa, H. Matsushita, Y. Terao, *Chemosphere* **2001**, *44*(5), 973. https://doi.org/10.1016/s0045-6535(00)00507-5

[3] Y. H. Tian, J. H. Baek, S. Y. Lee, C. G. Jang, *Synapse* **2010**, *64*(6), 432. https://doi.org/10.1002/syn.20746

[4] S. E. Elswefy, F. R. Abdallah, H. H. Atteia, A. S. Wahba, R. A. Hasan, *Int. J. Exp. Pathol.* **2016**, *97*(5), 369. https://doi.org/10.1111/iep.12207

[5] N. R. Gassman, *Environ. Mol. Mutagen*. **2017**, *58*(2), 60. https://doi.org/10.1002/em.22072

[6] L. A. Tse, P. M. Y. Lee, W. M. Ho, A. T. Lam, M. K. Lee, S. S. M. Ng, Y. He, K. S. Leung, J. C. Hartle, H. Hu, H. Kan, F. Wang, C. F. Ng, *Environ. Int.* **2017**, *107*, 1. https://doi.org/10.1016/j.envint.2017.06.012

[7] F. M. Huang, Y. C. Chang, S. S. Lee, Y. C. Ho, M. L. Yang, H. W. Lin, Y. H. Kuan, *Food Chem. Toxicol*. **2018**, *122*, 215. https://doi.org/10.1016/j.fct.2018.09.078

[8] J. R. Morrice, C. Y. Gregory-Evans, C. A. Shaw, *Sci. Rep*. **2018**, *8*(1), 4890. https://doi.org/10.1038/s41598-018-23018-w

[9] P. A. Olsvik, P. Whatmore, S. J. Penglase, K. H. Skjærven, M. Anglès d'Auriac, S. Ellingsen, *Front. Genet*. **2019**, *10*, 184. https://doi.org/10.3389/fgene.2019.00184

[10] S. S. Kim, K. S. Hwang, J. Y. Yang, J. S. Chae, G. R. Kim, H. Kan, M. H. Jung, H. Y. Lee, J. S. Song, S. Ahn, D. S. Shin, K. R. Lee, S. K. Kim, M. A. Bae, *Chemosphere* **2020**, *239*, 124751. https://doi.org/10.1016/j.chemosphere.2019.124751

[11] F. S. Vom Saal, C. Hughes, *Environ. Health Perspect*. **2005**, *113*(8), 926. https://doi.org/10.1289/ehp.7713

[12] B. S. Rubin, A. M. Soto, *Mol. Cell. Endocrinol*. **2009**, *304*(1-2), 55. https://doi.org/10.1016/j.mce.2009.02.023

[13] C. E. Talsness, A. J. Andrade, S. N. Kuriyama, J. A. Taylor, F. S. vom Saal, *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **2009**, *364*(1526), 2079. https://doi.org/10.1098/rstb.2008.0281

[14] T. Geens, L. Goeyens, A. Covaci, *Int. J. Hyg. Environ. Health* **2011**, *214*(5), 339. https://doi.org/10.1016/j.ijheh.2011.04.005

[15] B. S. Rubin, *J. Steroid Biochem. Mol. Biol*. **2011**, *127*, 127. https://doi.org/10.1016/j.jsbmb.2011.05.002

[16] K. A. Szychowski, A. K. Wójtowicz, *Postepy. Hig. Med. Dosw. (Online)*. **2013**, *67*, 499. https://doi.org/10.5604/17322693.1051001

[17] P. Charisiadis, X. D. Andrianou, T. P. Van der Meer, W. F. A. Den Dunnen, D. F. Swaab, B. H. R. Wolffenbuttel, K. C. Makris, J. V. Van Vliet-Ostaptchouk, *Sci. Rep*. **2018**, *8*(1), 8186. https://doi.org/10.1038/s41598-018-26498-y

[18] S. C. Nagel, J. J. Bromfield, *Endocrinology* **2013**, *154*(6), 1962. https://doi.org/10.1210/en.2013-1370

[19] G. W. Winston, *Comp. Biochem. Physiol. C* **1991**, *100*(1-2), 173. https://doi.org/10.1016/0742-8413(91)90148-m

[20] K. A. Kelly, C. M. Havrilla, T. C. Brady, K. H. Abramo, E. D. Levin, *Environ. Health Perspect*. **1998**, *106*(7), 375. https://doi.org/10.1289/ehp.98106375

[21] Z. A. Bault, S. M. Peterson, J. L. Freeman, *J. Appl. Toxicol*. **2015**, *35*(12), 1502. https://doi.org/10.1002/jat.3169

[22] C. H. Hsu, Z. H. Wen, C. S. Lin, C. Chakraborty, *Curr. Neurovasc. Res*. **2007**, *4*(2), 111. https://doi.org/10.2174/156720207780637234

[23] Z. Bencan, D. Sledge, E. D. Levin, *Pharmacol. Biochem. Behav*. **2009**, *94*(1), 75. https://doi.org/10.1016/j.pbb.2009.07.009

[24] L. Grossman, E. Utterback, A. Stewart, S. Gaikwad, K. M. Chung, C. Suciu, K. Wong, M. Elegante, S. Elkhayat, J. Tan, T. Gilder, N. Wu, J. Dileo, J. Cachat, A. V. Kalueff, *Behav. Brain Res*. **2010**, *214*(2), 277. https://doi.org/10.1016/j.bbr.2010.05.039

[25] D. L. Gebauer, N. Pagnussat, A. L. Piato, I. C. Schaefer, C. D. Bonan, D. R. Lara, *Pharmacol. Biochem. Behav*. **2011**, *99*(3), 480. https://doi.org/10.1016/j.pbb.2011.04.021

[26] A. Stewart, N. Wu, J. Cachat, P. Hart, S. Gaikwad, K. Wong, E. Utterback, T. Gilder, E. Kyzar, A. Newman, D. Carlos, K. Chang, M. Hook, C. Rhymes, M. Caffery, M. Greenberg, J. Zadina, A. V. Kalueff, *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2011**, *35*(6), 1421. https://doi.org/10.1016/j.pnpbp.2010.11.035

[27] J. Bouayed, H. Rammal, R. Soulimani, *Oxid. Med. Cell Longev*. **2009**, *2*(2), 63. https://doi.org/10.4161/oxim.2.2.7944

[28] S. H. Moussavi Nik, K. Croft, T. A. Mori, M. Lardelli, *Zebrafish* **2014**, *11*(3), 248. https://doi.org/10.1089/zeb.2013.0958

[29] OECD. OECD Guidelines for the Testing of Chemicals. Test No. 203: Fish, Acute Toxicity Test. Organization for Economic Co-operation and Development, Paris, France, **1992**.

[30] J. A. Kovrižnych, R. Sotníková, D. Zeljenková, E. Rollerová, E. Szabová, *Interdiscip. Toxicol.* **2014**, Mar *7*(1), 23. https://doi.org/10.2478/intox-2014-0004

[31] D. J. Finney, *Probit analysis*, Cambridge University Press, Cambridge, UK **1971**.

[32] R. J. Egan, C. L. Bergner, P. C. Hart, J. M. Cachat, P. R. Canavello, M. F. Elegante, S. I. Elkhayat, B. K. Bartels, A. K. Tien, D. H. Tien, S. Mohnot, E. Beeson, E. Glasgow, H. Amri, Z. Zukowska, A. V. Kalueff, *Behav. Brain Res*. **2009**, *205*(1), 38. https://doi.org/10.1016/j.bbr.2009.06.022

[33] R. E. Blaser, Y. M. Peñalosa, *Physiol. Behav*. **2011**, *104*(5), 831. https://doi.org/10.1016/j.physbeh.2011.07.029

[34] R. Mohanty, S. K. Das, M. Patri, *Neurotox. Res*. **2017**, *31*(4), 493. https://doi.org/10.1007/s12640-016-9694-5

[35] S. Bretaud, S. Lee, S. Guo, *Neurotoxicol. Teratol*. **2004**, *26*(6), 857. https://doi.org/10.1016/j.ntt.2004.06.014

[36] G. Dal Santo, G. M. Conterato, L. J. Barcellos, D. B. Rosemberg, A. L. Piato, *Neurosci. Lett*. **2014**, *558*, 103. https://doi.org/10.1016/j.neulet.2013.11.011
[37] R. L. Levine, D. Garland, C. N. Oliver, A. Amici, I. Climent, A. G. Lenz, B. W. Ahn, S. Shaltiel, E. R. Stadtman, *Methods Enzymol.* **1990**, *186*, 464. https://doi.org/10.1016/0076-6879(90)86141-h
[38] J. K. Callaway, P. M. Beart, B. Jarrott, *J. Pharmacol. Toxicol. Methods* **1998**, *39*(*3*), 155. https://doi.org/10.1016/s1056-8719(98)00022-7
[39] F. Tietze, *Anal. Biochem*. **1969**, *27*(*3*), 502. https://doi.org/10.1016/0003-2697(69)90064-5
[40] S. M. Miller, M. J. Moore, V. Massey, C. H. Williams Jr., M. D. Distefano, D. P. Ballou, C. T. Walsh *Biochemistry* **1989**, *28*(*3*), 1194. https://doi.org/10.1021/bi00429a037
[41] S. K. Das, I. Baitharu, K. Barhwal, S. K. Hota, S. B. Singh, *Neurosci. Lett*. **2016**, *612*, 231. https://doi.org/10.1016/j.neulet.2015.12.038
[42] I. Baitharu, V. Jain, S. N. Deep, S. Shroff, J. K. Sahu, P. K. Naik, G. Ilavazhagan, *PLoS One* **2014**, *9*(*10*), e105311. https://doi.org/10.1371/journal.pone.0105311
[43] S. Mehto, K. K. Jena, P. Nath, S. Chauhan, S. P. Kolapalli, S. K. Das, P. K. Sahoo, A. Jain, G. A. Taylor, S. Chauhan, *Mol. Cell* **2019**, *73*(*3*), 429. https://doi.org/10.1016/j.molcel.2018.11.018
[44] O. S. Spicer, T. T. Wong, N. Zmora, Y. Zohar, *PLOS One* **2016**, *11*(*6*), e0158141. https://doi.org/10.1371/journal.pone.0158141
[45] K. Stanic, G. Reig, R. J. Figueroa, P. A. Retamal, I. A. Wichmann, J. C. Opazo, G. I. Owen, A. H. Corvalán, M. L. Concha, J. D. Amigo, *Sci. Rep*. **2019**, *9*(*1*), 7131. https://doi.org/10.1038/s41598-019-43436-8
[46] J. Michałowicz, *Environ. Toxicol. Pharmacol*. **2014**, *37*(*2*), 738. https://doi.org/10.1016/j.etap.2014.02.003
[47] X. Li, M. Z. Sun, X. Li, S. H. Zhang, L. T. Dai, X. Y. Liu, X. Zhao, D. Y. Chen, X. Z. Feng, *Chemosphere* **2017**, *186*, 295. https://doi.org/10.1016/j.chemosphere.2017.07.164
[48] J. Wang, X. Wang, C. Xiong, J. Liu, B. Hu, L. Zheng, *Environ. Pollut*. **2015**, *206*, 275. https://doi.org/10.1016/j.envpol.2015.07.015
[49] R. E. Blaser, L. Chadwick, G. C. McGinnis, *Behav. Brain Res*. **2010**, *208*(*1*), 56. https://doi.org/10.1016/j.bbr.2009.11.009
[50] J. B. Schulz, J. Lindenau, J. Seyfried, J. Dichgans, *Eur. J. Biochem*. **2000**, *267*(*16*), 4904. https://doi.org/10.1046/j.1432-1327.2000.01595.x
[51] S. Crockett, M. Clarke, S. Reeves, B. Sims, *NeuroReport* **2011**, *22*(*12*), 598. https://doi.org/10.1097/WNR.0b013e3283494359
[52] V. Lobo, A. Patil, A. Phatak, N. Chandra, *Pharmacogn. Rev*. **2010**, *4*(*8*), 118. https://doi.org/10.4103/0973-7847.70902
[53] Y. Zhou, Z. Wang, M. Xia, S. Zhuang, X. Gong, J. Pan, C. Li, R. Fan, Q. Pang, S. Lu, *Environ. Pollut*. **2017**, *229*, 40. https://doi.org/10.1016/j.envpol.2017.05.043
[54] W. Zhang, C. Sun, Y. Shao, Z. Zhou, Y. Hou, A. Li, *Sci. Rep*. **2019**, *9*(*1*), 254. https://doi.org/10.1038/s41598-018-36538-2
[55] J. Jankovic, S. Chen, W. D. Le, *Prog. Neurobiol*. **2005**, *77*, 112. https://doi.org/10.1016/j.pneurobio.2005.09.001
[56] B. Kadkhodaei, T. Ito, E. Joodmardi, B. Mattsson, C. Rouillard, M. Carta, S. Muramatsu, C. Sumi-Ichinose, T. Nomura, D. Metzger, P. Chambon, E. Lindqvist, N. G. Larsson, L. Olson, A. Björklund, H. Ichinose, T. Perlmann, *J. Neurosci*. **2009**, *29*(*50*), 15923. https://doi.org/10.1523/JNEUROSCI.3910-09.2009
[57] O. Saucedo-Cardenas, J. D. Quintana-Hau, W. D. Le, M. P. Smidt, J. J. Cox, F. De Mayo, J. P. Burbach, O. M. Conneely, *Proc. Natl. Acad. Sci. U. S. A*. **1998**, *95*(*7*), 4013. https://doi.org/10.1073/pnas.95.7.4013
[58] K. Messmer, M. P. Remington, F. Skidmore, P. S. Fishman, *Int. J. Dev. Neurosci*. **2007**, *25*(*1*), 29. https://doi.org/10.1016/j.ijdevneu.2006.11.003
[59] L. Stefanis, *Cold Spring Harb. Perspect. Med*. **2012**, *2*(*2*), a009399. https://doi.org/10.1101/cshperspect.a009399
[60] Y. Zhu, J. Zhang, Y. Zeng, *CNS. Neurol. Disord. Drug. Targets*. **2012**, *11*(*4*), 350. https://doi.org/10.2174/187152712800792901
[61] D. J. Gelb, E. Oliver, S. Gilman, *Arch. Neurol*. **1999**, *56*(*1*), 33. https://doi.org/10.1001/archneur.56.1.33
[62] L. Xin, Y. Lin, A. Wang, W. Zhu, Y. Liang, X. Su, C. Hong, J. Wan, Y. Wang, H. Tian, *Environ. Toxicol. Pharmacol*. **2015**, *40*(*2*), 524. https://doi.org/10.1016/j.etap.2015.08.002
[63] T. Iso, T. Watanabe, T. Iwamoto, A. Shimamoto, Y. Furuichi, *Biol. Pharm. Bull*. **2006**, *29*(*2*), 206. https://doi.org/10.1248/bpb.29.206

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** P. K. Sahoo, S. Aparna, P. K. Naik, S. B. Singh, S. K. Das, *J. Biochem. Mol. Toxicol*. **2021**, e22873. https://doi.org/10.1002/jbt.22873