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Neurodevelopmental and behavioural effects of waterborne selenite in larval zebrafish (*Denio rerio*)

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1. Introduction

Selenium (Se) is an essential trace element but can become extremely toxic to fishes at concentrations that exceed their physiological optimum (Janz et al., 2010; Hamilton, 2004; Lemly, 2004). Selenium plays a crucial role in various biological processes, including brain function, thyroid hormone regulation, and antioxidative response (Labunskyy et al., 2014; Mullur et al., 2014; Mehdi et al., 2013; Richardson, 2005; Chen and Berry, 2003). However, the narrow margin between the essentiality and toxicity of Se makes it a double-edged sword, especially for oviparous animals like fish (Janz et al., 2010). Toxic effects in some fishes are observed when dietary Se concentrations exceed 3 mg/g dry weight (dw), compared to its essential range of 0.1–0.3 mg/g dw (Thomas and Janz, 2011).

Anthropogenic activities, such as agricultural runoff, coal combustion, metal mining, and oil refining, have significantly redistributed Se in the environment, leading to elevated concentrations, particularly in several regions across North America (He et al., 2018). Freshwater systems contaminated by Se are often dominated by dissolved inorganic Se oxyanions, particularly selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}) (Simmons and Wallschlager, 2005). Concentrations of these species can vary widely, ranging from 1 to 10 μg/L in natural waters (Sohrin and Bruland, 2011), with higher levels found in certain regions, such as Se-rich areas in China, where inorganic Se species constitute up to 99.5% of total Se (Qiu et al., 2013). In highly contaminated aquatic systems, dissolved Se concentrations have been reported to be in the range of 11.44–341 μg/L (Kelly et al., 2018; Haque et al., 2016; Bajaj et al., 2011).

Selenite is acutely toxic to fishes, as demonstrated by several *in vitro* studies with primary cell cultures and cell lines as well as *in vivo* studies with whole fishes (Mo et al., 2021; Masse et al., 2013; Selvaraj et al., 2012; Miller and Hontela, 2011; Misra and Niyogi, 2009). Moreover, chronic aqueous exposure to elevated levels of selenite has been reported to impair growth, hepatic and reproductive functions in adult fishes (Cheng et al., 2022, 2023; Chernick et al., 2016; Penglase et al., 2014; Wiseman et al., 2011). However, much less is known about the toxicity of selenite in the early life stages of fishes beyond its reported negative impact on larval survival and morphology in larval zebrafish (*Danio rerio*) (Ma et al., 2012).

Selenite is also known to induce neurobehavioural toxicity in humans at supra-nutritional levels (Vinceti et al., 2014). However, most evidence of the neurobehavioural toxicity of Se in fishes stems from studies involving selenomethionine (Se-Met), a prevalent organic form of Se in aquatic environments. Chronic exposure to Se-Met has been suggested to impair several behavioural responses in adult zebrafish, including learning and memory functions, social and antipredator behaviours, and anxiogenic behaviours, by inducing oxidative stress and dysregulation of monoaminergic (dopaminergic and serotonergic) neural signalling pathways in the brain (Attaran et al., 2019, 2020; Naderi et al., 2017, 2018a). Moreover, similar neurobehavioural disruptions were also observed in the adult offspring of zebrafish maternally exposed to Se-Met, indicating that neurobehavioural effects of Se can persist beyond the parental generation (Attaran et al., 2021; Naderi et al., 2018b). However, much of our current knowledge of the neurobehavioral effects of Se in fishes is based on studies with adult life stages, and very little is known about how Se affects the neurodevelopment and behaviour in early life stages.

Early life stages are critical windows of susceptibility when exposure to contaminants like Se can disrupt neural development and programming, leading to long-term behavioural deficits. Selenite is the most toxic inorganic form of Se (Masse et al., 2013), and it has been recently found to induce apoptosis and impair development and anxiogenic behaviours in larval zebrafish (Hariharan et al., 2024). However, this study used a Se exposure range (~200–5000 μg/L), which is well above the dissolved Se levels observed in contaminated aquatic ecosystems and thus has limited ecological relevance. It is also to be noted that larval fish display other more complex and ecologically important behaviours, such as shoaling and learning, and how these behaviours are impacted

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by developmental Se exposure is currently unknown. Moreover, the cellular and molecular mechanisms underlying the neurobehavioural effects of developmental exposure to Se remain poorly understood. Behavioural impairments in larval fishes, such as altered learning or predator avoidance, can reduce their chances of survival, thereby threatening the long-term health of aquatic ecosystems (Rice and Barone, 2000).

The present study investigated the developmental and neurobehavioural toxicity of selenite in larval zebrafish. Zebrafish were chosen as the model organism due to their high fecundity, rapid development, and well-established use in toxicological and neurobehavioural studies (Panula et al., 2010). We hypothesized that environmentally relevant concentrations of Se (as selenite) would induce developmental and behavioural abnormalities *via* oxidative stress and dysregulation of monoaminergic neurotransmission systems. To test this hypothesis, zebrafish embryos were exposed to Se concentrations of 0 (control), 10, 50 and 100 μg/L (as selenite) from 0 to 30 days post-fertilization (dpf), and subsequently, larval survival and development, anxiogenic, social and exploratory behaviours, biochemical markers of oxidative stress, and expression of genes involved in neurodevelopment and monoaminergic signalling were analyzed.

2. Materials and methods

2.1. Chemicals and reagents

Sodium selenite (Na_2SeO_3; CAS No. 10102-18-8; purity: 99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). A stock solution with a concentration of 50 mg Se/L was prepared by dissolving sodium selenite in ultrapure water and stored at 4 °C. Experimental solutions were prepared by diluting the stock solution with either E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$) or dechlorinated Saskatoon municipal water (total hardness: 150 mg/L as $CaCO_3$; total alkalinity: 120 mg/L as $CaCO_3$; and pH: 7.5–8.0.). Methanesulfonate-222 (MS-222) and 1-phenyl-2-thiourea (PTU) were also obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in this experiment were of analytical grade.

2.2. Zebrafish maintenance and eggs collection

A total of 30 adult zebrafish (20 females and 10 males, 7 months old) were obtained from our breeding colony housed in the Collaborative Sciences Research Building at the University of Saskatchewan. The fish were maintained in a flow-through system with dechlorinated Saskatoon municipal water, which had a total hardness of 150 mg/L as $CaCO_3$, alkalinity of 120 mg/L as $CaCO_3$, and pH of 7.5–8.0. Water temperature was maintained at 27 °C, and the photoperiod was set to 14:10 h light and dark. Fish were fed Nutrafin Max flakes (Germany) in the morning and Hikari BIO-PURE® frozen bloodworms (California, USA) in the afternoon daily. Sexually mature fish without deformities or signs of disease were selected for breeding. Two females and one male were placed in each breeding tank, separated by a partition overnight, which was removed the next morning to facilitate breeding. Spawning was induced with the onset of light, and after 30 min, embryos were collected and examined under a dissecting microscope. Unfertilized and dead embryos were removed. Breeding tanks were equipped with a perforated structure at the bottom to protect eggs from predation by the adult fish. A total of 800 normally developing blastula stage embryos (4 hours post-fertilization, hpf) were selected under a stereomicroscope and subjected to various treatments to investigate the developmental toxicity of Se.

2.3. Experimental design

Blastula stage (4 hpf) embryos were randomly assigned to 90 mm Petri dishes containing 50 mL of Se exposure solution at concentrations of 0, 10, 50 and 100 μg/L for 5 days post fertilization (dpf). The nominal concentrations were verified by measuring the total Se concentrations using Atomic Absorption Spectrometry (AAnalyst 800, PerkinElmer Life and Analytical Sciences, USA) (see supplementary file for details). Each treatment group consisted of four replicates with 50 embryos per replicate. The Se concentrations used in the present study were comparable to those found in Se-contaminated natural waters (Cheng et al., 2022, 2023). Dead embryos and egg chorions were regularly removed from the exposure media. At 5 dpf, 25 zebrafish larvae were selected and transferred into rearing tanks containing 2 L of Se exposure solution at concentrations of 0, 10, 50 and 100 μg/L for 30 days. Each treatment group had four replicates, and each replicate contained 25 larvae. The present study employed a semi-static exposure regime, which involved daily refreshing 50% of the exposure solution with the corresponding Se concentrations. Embryos from the same batch were used for both treatment and control groups in each experiment, which was replicated four times.

After yolk sac absorption, zebrafish larvae were initially fed GEMMA Micro 75 as a starter diet twice daily for the first three days. Subsequently, they were provided Artemia twice daily throughout the experimental period. The larvae were maintained under a photoperiod of 14 h light and 10 h dark at a temperature of 28 °C. At 5 dpf, endpoints including hatching rate, deformity rate, and larval survivability were determined using the standard formulas (see supplementary files for details). In addition, DCF-DA (2′,7′-dichlorodihydrofluorescein diacetate) and AO (acridine orange) staining of the 5 dpf larvae were also performed to determine ROS (reactive oxygen species) levels and cell apoptosis, respectively. To suppress pigment expression during the evaluation of DCF-DA and AO staining, 0.003% (w/v) 1-phenyl-2-thiourea (PTU) was added to the exposure solutions. However, no PTU treatment was conducted for assessing the developmental parameters *via* bright-field microscopy and behavioural assays described below at any life stages of larval zebrafish.

Different behaviours were evaluated at different life stages of larval zebrafish. At 5 dpf, the light-dark preference test and thigmotactic behaviour were conducted to assess anxiety levels due to Se exposure. Following those behavioural assessment, fish larvae were immediately placed in liquid RNAlater and stored at −80 °C for gene expression analysis by quantitative real-time polymerase chain reaction (qRT-PCR). Additional behavioural analyses included, the social preference test at 21 dpf to evaluate sociality, and the novel object recognition test at 30 dpf to measure exploration levels. For each behavioural study, n = 32 per treatment group was maintained. In addition, the total length (cm) and body weight (mg) of zebrafish larvae were measured at 30 dpf, using 20 individuals from each treatment group. All procedures in this study were approved by the University of Saskatchewan Animal Research Ethics Board (protocol no. 20230069).

2.4. Behavioural assays

2.4.1. Light-dark preference test

Zebrafish sensory-motor reflexive networks develop and mature between 24 and 27 h post-fertilization (hpf), with sensory reflexes to light becoming apparent around this time. At 5 dpf, zebrafish larvae exhibit autonomous locomotion and exploration. The reflexive movement test, also known as the spontaneous light/dark cycle test, was employed to investigate the effect of Se on the development of motor responses in embryonic zebrafish. Larvae were placed individually in each well of a transparent 6-well plate and allowed to acclimate for 20 min. Following acclimatization, the larvae were exposed to cycles of 5 min of visible light and 5 min of darkness for a total duration of 20 min (Fig. S1). The dark phase was created by using infrared light, and their movements were video recorded using a GoPro Hero4 camera (v05.00). Maximum speed and distance travelled during dark-to-light transitions were analyzed from the recordings using a customized *Python* software, which is capable of tracking 30 frames/second (Irons et al., 2010; Xu et al., 2022). Key parameters assessed included total distance travelled

(mm), activity counts reflecting movement frequency, and maximum speed during light and dark phases.

2.4.2. Thigmotaxis test

Thigmotaxis is a reliable indicator of anxiety, as it measures an animal's preference for staying in sheltered areas rather than open spaces. To conduct this behavioural test, 5 dpf individual larva was placed in each well of a 6-well plate. After a 10-min acclimation period, the movements of the larva were video recorded for an additional 10 min using a GoPro Hero4 camera (v05.00). The movements were analyzed from the recordings using a customized *Python* software capable of tracking 30 frames/second. Thigmotaxis was determined by measuring the percentage of the total distance travelled by the larva in the outer zone of the wells and the percentage of time spent in this outer zone. The outer zone was defined as the area within 8 mm from the border of each well (Fig. S2) (Han et al., 2021; Merola et al., 2021).

2.4.3. Social preference test

For this test, the observational tank was divided into three compartments using two glass dividers. One of the side compartments (6 cm wide) served as the 'conspecific box,' which housed conspecifics, while the opposite compartment remained empty and was designated as the 'empty box' (Fig. S3). The central compartment (18 cm wide) was further divided into three zones: the conspecific zone (6 cm) adjacent to the conspecific box, the empty zone (6 cm) adjacent to the empty box, and the central zone (6 cm) situated between the conspecific and empty zones. At 21 dpf, five individuals from each treatment group were transferred to the conspecific box. A focal fish from the same treatment group was then placed in the central zone. Following a 1-min adaptation period, the time spent by the focal fish in each zone was video recorded for 10 min using a GoPro Hero4 camera (v05.00). The percentage of total time spent near the conspecific box was calculated as a measure of social preference from the footage using a customized Python software capable of tracking 30 frames/second. To minimize bias, the position of the conspecific box (left or right) was alternated for 50% of the total replicates ($n = 32$) per treatment.

2.4.4. Novel object recognition test

At 25 dpf, larvae from both the control and treatment groups were habituated to a custom-made empty chamber (9 cm length x 9 cm width x 6 cm height) for 8 min, twice daily, over 5 consecutive days (Fig. S4). At 30 dpf, individual larvae were trained for object recognition by introducing them to two identical objects (two green round-shaped LEGO® pieces) within the chamber for 8 min. Following an interval of 1.5 h, each larva was reintroduced to the chamber, where one of the objects had been replaced with a new object (a red round-shaped LEGO® piece). The larvae were given another 8 min to explore the objects, and their behaviour was video recorded using a GoPro Hero4 camera (v05.00). The tank was cleaned, and the water was changed after each trial. The exploration ratio was calculated from the footage using the formula: time spent with the novel object/(time spent with the novel object + time spent with the familiar object). A score greater than 0.5 was considered indicative of a preference for the novel object, which is interpreted as normal behaviour. Video tracking and analysis were conducted using a customized *Python* software capable of tracking 30 frames/second.

2.5. Determination of ROS levels

The production of intracellular ROS in zebrafish larvae upon exposure to different environmentally relevant concentrations of Se was determined with an oxidation-sensitive fluorescent probe DCF-DA. To analyze ROS levels, 10 live 5 dpf zebrafish embryos from each treatment group were washed three times with 1 × PBS (pH 7.4) and then incubated with 20 μg/mL DCF-DA solution for 2 h in the dark. Subsequently, the embryos were washed again three times with 1 × PBS (pH 7.4) and were positioned on glass depression slides in lateral recumbency for imaging. A solution of 3.5% methylcellulose was used to immobilize the larvae during imaging. ROS levels were observed using a Zeiss AxioPlan fluorescence microscope, and fluorescence intensity was quantified using *ImageJ* (Yang et al., 2021). The excitation wavelength was set to 450–490 nm, and the emission wavelength was 510–520 nm. Fluorescence quantification was determined using the corrected total cell fluorescence (CTCF) formula:

$$\text{CTCF} = \text{integrated density} - (\text{area of selected cell} \times \text{mean fluorescence of background reading})$$

2.6. Determination of cell apoptosis

Acridine orange (AO) is a fluorochrome dye capable of intercalating into nucleic acids to identify cell apoptosis (Lite et al., 2022). As described for DCF-DA staining, ten 5 dpf larvae from each Se treatment were washed three times with PBS, incubated in a 10 μg/mL AO solution for 30 min in the dark, and then washed again three times with PBS. Larvae were then immobilized in 3.5% methylcellulose solution for imaging. A fluorescent stereomicroscope was utilized to take images of the larvae, and the fluorescence intensity of whole-mount larvae was measured with *ImageJ* software using the same formulas as described for ROS determination.

2.7. RNA extraction, cDNA synthesis and quantitative real-time PCR analysis

Based on their involvement in neurodevelopment and neural signalling pathways, apoptotic, and antioxidative functions, the expression of the following genes was assessed by qRT-PCR: dopaminergic genes (*mao, th1, robo, opta, sncgb*); serotonergic genes (*5ht1ab, 5ht2c, tph2, pet1, serta*); cholinergic genes (*sox, isll, insm1a*); genes related to neurogenesis (*huc, bdnf*); genes related to antioxidant response (*nrf2a, nrf2b, cu-zn-sod, mn-sod, gpx*), and a gene involved in apoptotis (*caspase3*). Total RNA was extracted from pooled samples of 10 zebrafish larvae per treatment using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. RNA concentrations and purity were assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA), with absorbance measured at 260 and 280 nm. Samples with a 260/280 nm ratio between 1.9 and 2.0 were selected for further analysis. Subsequently, 1 μg of total RNA was used to synthesize cDNA utilizing the QuantiTect Reverse Transcription Kit (Qiagen, Germany). The qRT-PCR primers used are listed in Table S1. A 20 μL PCR reaction mixture was prepared, consisting of 10 μL SYBR Green PCR Master Mix (SensiFast, Bioline, USA), 2 μL cDNA, 0.8 μL each of forward and reverse gene-specific primers (Table S1), and 6.4 μL nuclease-free water. The initial denaturation was performed at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min, using an ABI QuantStudio 6 (Applied Biosystems, USA). β-actin was used as the housekeeping gene due to its stable expression in zebrafish embryos (Casadei et al., 2011). Relative quantification of target genes was analyzed using the $2\hat{}{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.8. Statistical analysis

All data were analyzed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA). Normality and homoscedasticity of the data were assessed using the Shapiro-Wilk and Levene tests, respectively. For data meeting the assumption of homogeneity of variances, a one-way analysis of variance (ANOVA) was employed to evaluate the effects of the main factor (i.e., Se) on the measured parameters followed by a Dunnett's post-hoc test to identify differences among treatments. Results are presented as mean ±

standard error of the mean (SEM), with statistical significance defined as $p < 0.05$. Alpha values were two-tailed and set at 0.05. In instances where data exhibited heteroscedasticity, Kruskal-Wallis tests were utilized instead of parametric ANOVAs, followed by a Dunn's post-hoc tests.

3. Results

3.1. Selenium exposure concentrations

The exposure solutions from different Se treatments were collected at the beginning of exposure and just before their renewal at 24 h and analyzed for total dissolved Se concentrations. The variation between the nominal and measured Se concentration in all treatments was less than 5% (see the Supplementary section for details), therefore, the different treatment groups have been reported here based on the nominal Se concentrations.

3.2. Mortality, hatching rate, deformities, and growth of zebrafish larvae

To assess the developmental toxicity of Se on zebrafish embryos and larvae, survival rate, hatching success, larval deformities, and growth were evaluated. Zebrafish embryos began hatching between the second and third days of exposure. Statistical analysis indicated no significant differences in cumulative hatchability, survival rate, or incidence of larval deformities between the control and Se-treated groups ($p > 0.05$). The cumulative hatching and survival rates were consistently around or above 80% across the treatments and control group (Fig. S5). The percentage of larval deformities was approximately 4% in the control group, compared to 6–8% in the three Se-exposure groups (Fig. S5). At 30 dpf, the total length (cm) was significantly reduced in Se-exposed groups compared to the control group (Kruskal-Wallis test: $\chi^2_3 = 14.02$, $p = 0.001$; Fig. 1). Specifically, larvae exposed to 50 μg/L and 100 μg/L Se showed a significant reduction in length compared to the control (Dunn's multiple comparisons test: $p = 0.012$ and $p = 0.01$, respectively). Similarly, body weight (mg) was significantly decreased in the 50 μg/L and 100 μg/L Se -exposed groups compared to the control group (One-way ANOVA followed by Dunnett's multiple comparisons test: $F_{3,76} = 8.28$, $p < 0.001$; $p < 0.001$ and $p = 0.001$, respectively; Fig. 2).

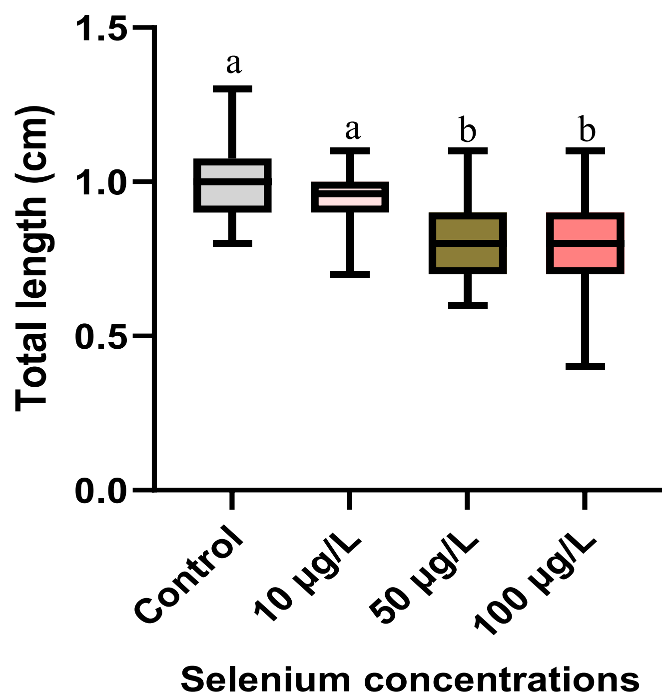

Fig. 1. Box plot indicates median of total length (cm) in 30 dpf larvae exposed to different concentrations of waterborne selenium. The horizontal center line in the boxes represents the median. The box reflects the median contained in the first and third quartiles (Q1 and Q3), with whiskers extending to minimum and maximum values. Different letters above data box denote a significant difference relative to control group at $p < 0.05$ (n = 4 replicates of 20 larvae). dpf-days post fertilization.

3.3. Behavioural performance

3.3.1. Light-dark reflexive response

In light and dark reflexive behaviour assay with 5 dpf zebrafish larvae, the total distance travelled (mm) were found to be significantly reduced in Se-treated groups compared to the control (Kruskal-Wallis test: $\chi^2_3 = 9.95$, $p = 0.02$; Fig. 3a) with marked reduction at 100 μg/L Se exposure (Dunn's multiple comparisons test: $p = 0.02$). Similarly, transition numbers per minute were significantly decreased in the 100 μg/L Se -exposed group compared to the control (Kruskal-Wallis test followed by Dunn's multiple comparisons test: $\chi^2_3 = 15.99$, $p = 0.001$; $p = 0.0002$, Fig. 3b. Furthermore, Kruskal-Wallis test also revealed that in both light and dark conditions, the average maximum speed (mm/s) was significantly reduced in 100 μg/L Se-treated group compared to the control (all $p < 0.05$) (Dunn's multiple comparisons test: $p = 0.02$ for both light and dark, Fig. 3c and d, respectively).

3.3.2. Thigmotaxis test

Thigmotaxis behaviour was assessed at 5 dpf by deriving the distance travelled in inner and outer zone and percent time spent in inner and outer zone of the assay chamber. There was a significant effect of Se on the percent time spent in both inner (One-way ANOVA: $F_{3,142} = 7.89$, $p < 0.001$; Fig. 4a) and outer zone (One-way ANOVA: $F_{3,142} = 8.43$, $p < 0.001$; Fig. 4b) compared to the control. Dunnett's multiple comparisons test revealed that 100 μg/L Se-treated group spent significantly more time (~63% of total time) in the inner zone ($p < 0.05$) and less time (~37%) in the outer zone ($p < 0.05$). Representative heatmaps indicating the movement and location of the larvae during behavioural

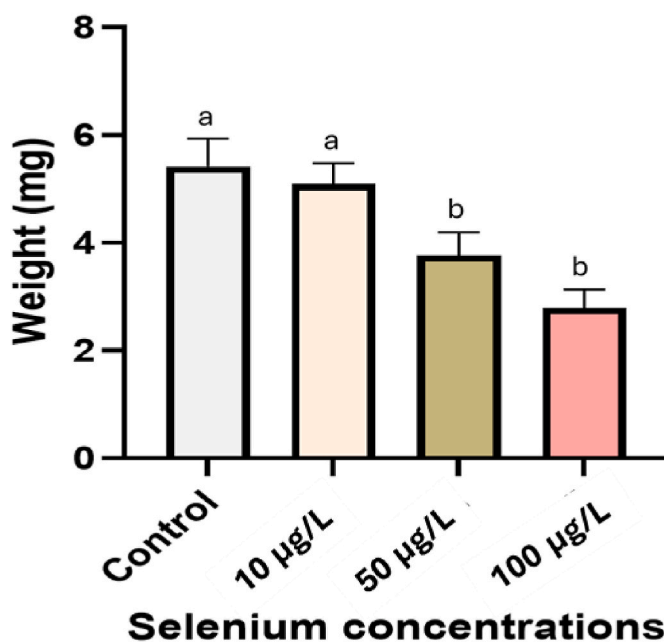

Fig. 2. Body weight (mean ± SEM) of zebrafish larvae upon exposure to different concentrations of waterborne selenium at 30 dpf. Different letters above data bars denote a significant difference relative to control group at $p < 0.05$. (n = 4 replicates of 20 larvae). dpf-days post fertilization.

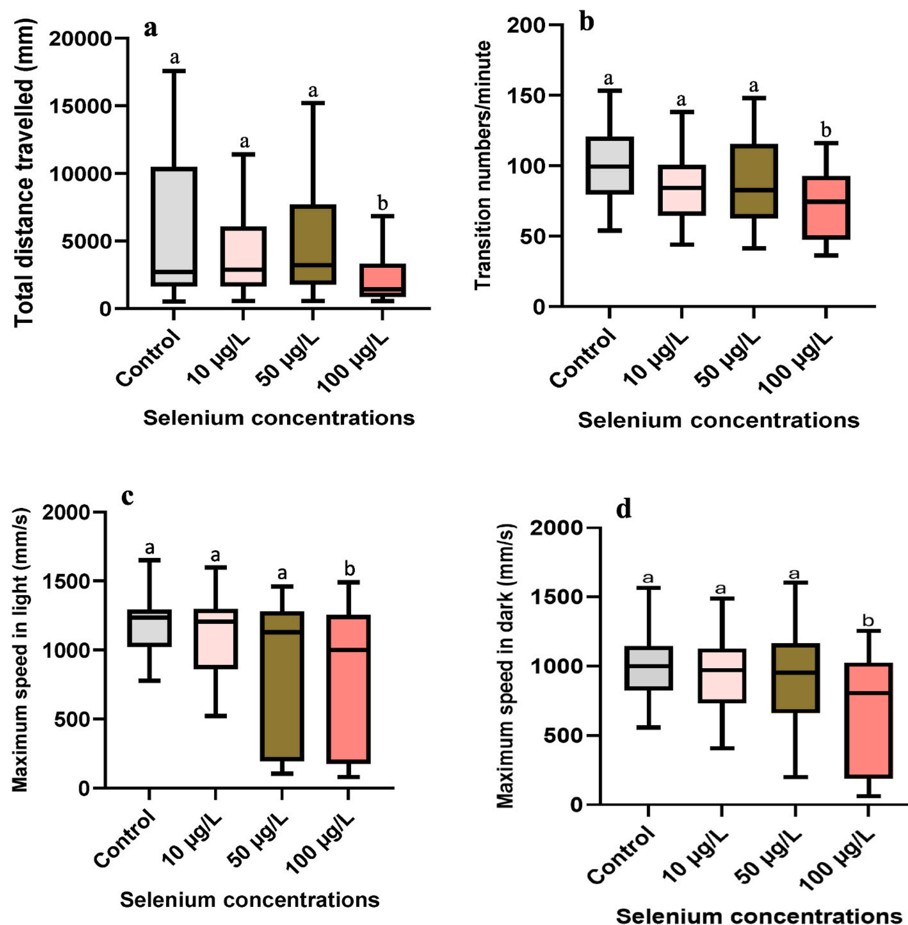

Fig. 3. Box plot indicates the effects of waterborne selenium exposure on the median total distance travelled (Fig. 3a); transition numbers per minute (Fig. 3b); maximum swimming speed in light (Fig. 3c), and dark (Fig. 3d) phases in larval zebrafish. The horizontal center line in the boxes represents the median. The box reflects the median contained in the first and third quartiles (Q1 and Q3), with whiskers extending to minimum and maximum values. Different letters above data box denote a significant difference relative to control group at $p < 0.05$ (n = 4 replicates of 28–38 larvae).

probing are shown in Fig. 4c. Furthermore, 50 and 100 µg/L Se-exposure groups showed significantly lower activity in the outer zone (Kruskal-Wallis test followed by Dunn's multiple comparisons test: $\chi^2_3 = 11.26$, $p = 0.01$; $p = 0.03$ and $p = 0.008$ respectively; Fig. 5a) whereas 100 µg/L showed higher activity in the inner zone (Kruskal-Wallis test followed by Dunn's multiple comparisons test: $\chi^2_3 = 9.26$, $p = 0.03$; $p = 0.01$; Fig. 5b).

3.3.3. Social preference test

Exposure to Se significantly affected the total time that 21 dpf zebrafish larvae spent near their conspecifics (Kruskal-Wallis test: $\chi^2_3 = 13.80$, $p = 0.003$; Fig. 6a and b). Fish exposed to the 50 and 100 µg/L Se concentrations spent significantly less time (~62%) near their conspecifics compared to the control fish (~82%) (Dunn's multiple comparisons test: $p = 0.001$ and $p = 0.01$ respectively), while there was no significant difference between the zebrafish larvae that were exposed to the 10 µg/L Se concentration and the control ($p = 0.06$; Fig. 6a).

3.3.4. Object recognition test

The novel object recognition ratio was found to be significantly influenced by different concentrations of Se at 30 dpf larval zebrafish (One way ANOVA: $F_{3,103} = 13.865$; $p < 0.001$; Fig. 7a and b). Larvae in 50 and 100 µg/L Se exposure groups were found to exhibit significantly lower exploratory response to the novel object compared to the larvae in control and 10 µg/L Se treatments ($p < 0.05$). The exploration tendency was 1.55 times lower in larvae exposed to 50 and 100 µg/L Se treatment groups compared to those exposed to 10 µg/L Se as well as the control larvae.

3.4. ROS production and apoptosis assay

At 5 dpf, accumulation of ROS levels was observed throughout the entire body, with a pronounced increase in the head region in larvae exposed to Se (Fig. 8). The Kruskal-Wallis test revealed a significant increase in fluorescence intensity in the Se -exposed groups ($\chi^2_3 = 11.23$, $p < 0.001$). Dunn's multiple comparisons test further confirmed a significant increase in fluorescence intensity in larvae treated with 100 µg/L of Se compared to the control ($p < 0.001$). It is to be noted that ROS production also increased in 10 and 50 µg/L of Se treatments, although it was not significantly different compared to the control group. Similarly, abundance of apoptotic cells was found to be significantly increased in larvae treated with different concentrations of Se (Kruskal-Wallis test: $\chi^2_3 = 8.74$, $p < 0.001$; Fig. 9). Notably, larvae exposed to 100 µg/L Se exhibited a significantly higher number of apoptotic cells compared to the control group (Dunn's multiple comparisons test: $p < 0.001$).

3.5. Gene expressions at 5 dpf zebrafish larvae

3.5.1. Expression of antioxidants and apoptotic genes

A significant alteration in the transcript levels of *Mn-sod* and *Cu/Zn-sod* genes was observed in the zebrafish larvae following exposure to waterborne Se. A Kruskal-Wallis test revealed a significant effect on *Mn-sod* expression ($\chi^2_4 = 15.91$, $p = 0.001$), while *Cu/Zn-sod* expression was also found to be significantly affected (One-way ANOVA: $F_{3,17} = 9.35$, p

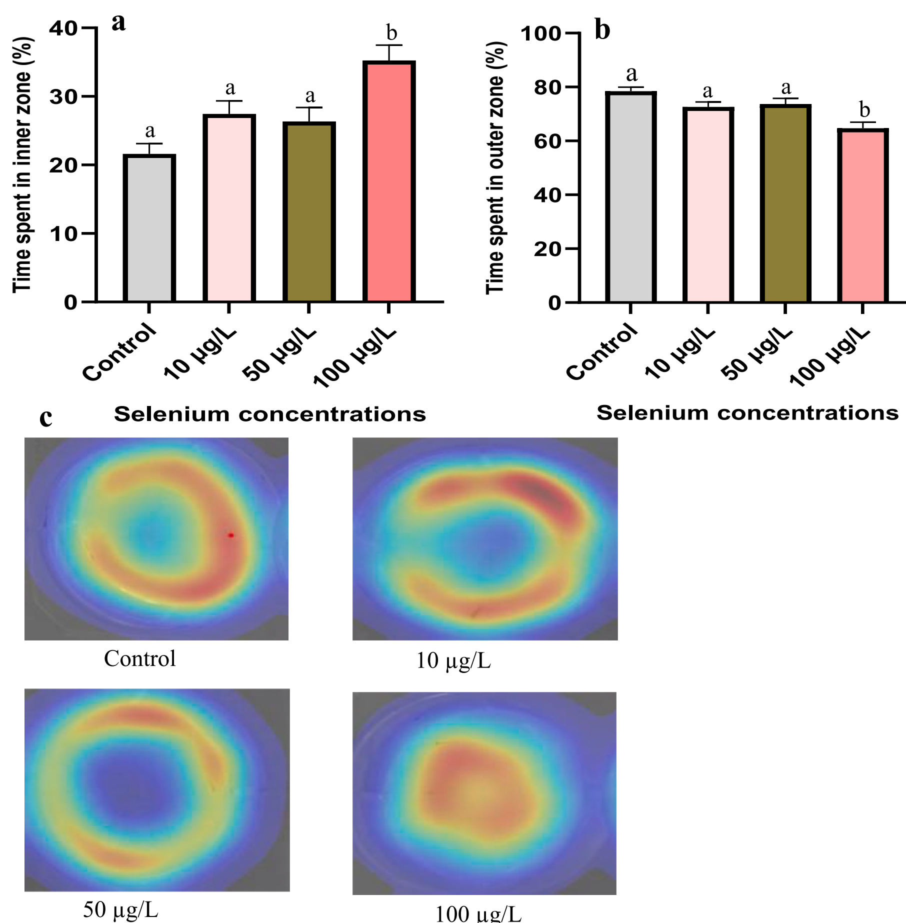

Fig. 4. Percent time spent (mean ± SEM) in inner (Fig. 4a) and outer (Fig. 4b) zone of zebrafish larvae upon exposure to different concentrations of waterborne selenium at 5 dpf. Different letters above data bars denote a significant difference relative to control group at $p < 0.05$. (n = 4 replicates of 32–40 larvae). dpf-days post fertilization. Heatmaps (Fig. 4c) representing the thigmotaxis behaviour in zebrafish across different concentrations of selenium. Warmer colours (red, yellow) represent regions where zebrafish spent more time. Cooler colours (blue) represent areas of less activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

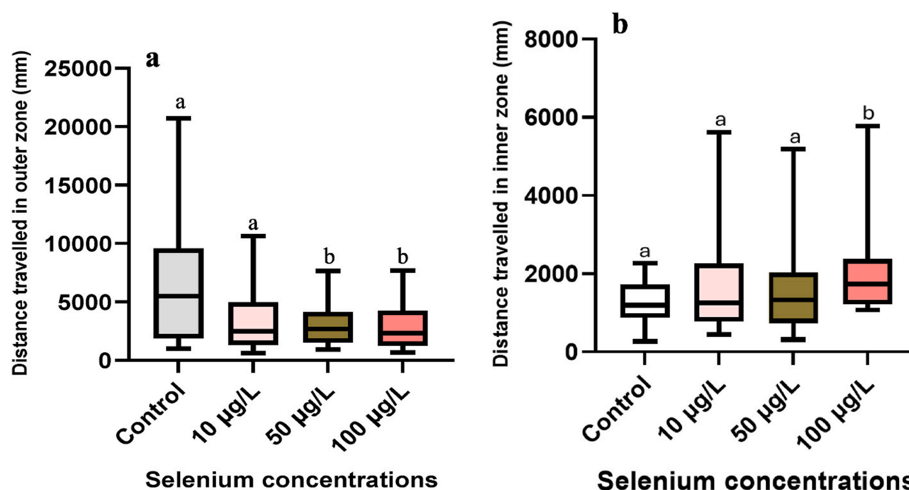

Fig. 5. Box plot indicates the effects of waterborne selenium exposure on the median distance travelled in outer (Fig. 5a) and inner (Fig. 5b) zone of larval zebrafish. The horizontal center line in the boxes represents the median. The box reflects the median contained in the first and third quartiles (Q1 and Q3), with whiskers extending to minimum and maximum values. Different letters above data box denote a significant difference relative to control group at $p < 0.05$ (n = 4 replicates of 32–40 larvae).

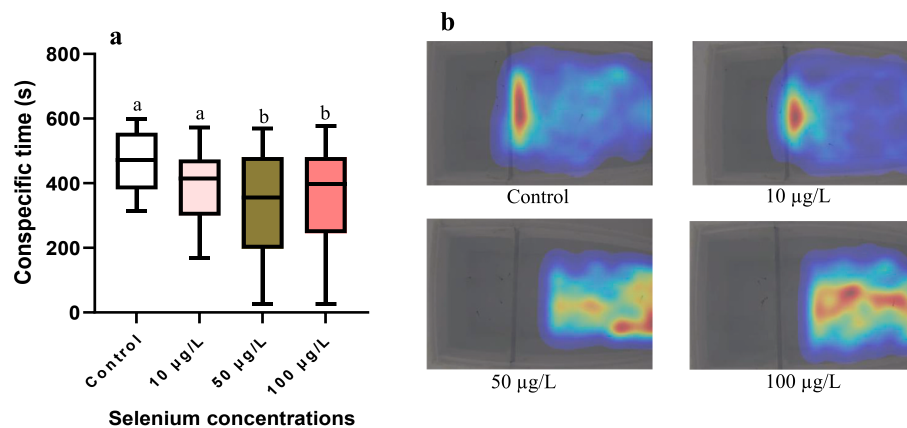

Fig. 6. Box plot indicates the effects of waterborne selenium exposure on the social preference behaviour of larval zebrafish. The horizontal center line in the boxes represents the median. The box reflects the median contained in the first and third quartiles (Q1 and Q3), with whiskers extending to minimum and maximum values. Different letters above data box denote a significant difference relative to control group at $p < 0.05$ (n = 4 replicates of 26–32 larvae). Heatmaps representing the social preference behaviour in zebrafish across different concentrations of selenium. Warmer colours (red, yellow) represent regions where zebrafish spent more time. Cooler colours (blue) represent areas of less activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

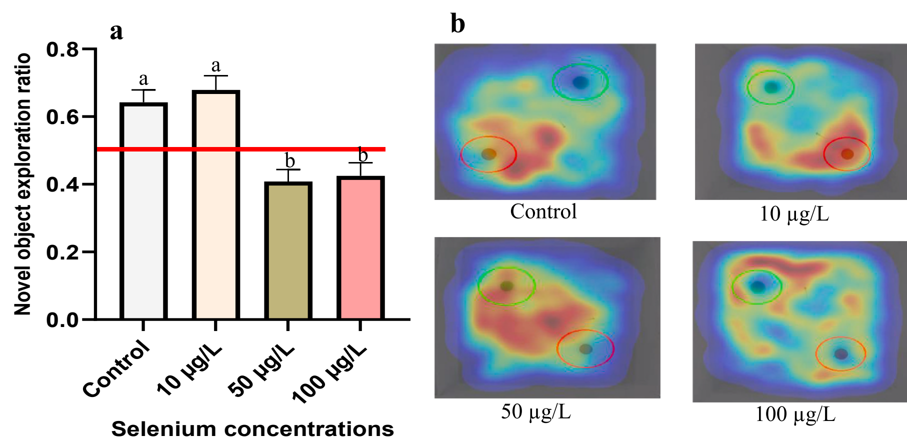

Fig. 7. Novel object exploration ratio (mean ± SEM) of zebrafish larvae upon exposure to different concentrations of waterborne selenium at 30 dpf. Different letters above data bars denote a significant difference relative to control group at $p < 0.05$. (n = 4 replicates of 24–28 larvae). dpf-days post fertilization. Heatmaps (Fig. 7b) representing the novel object recognition behaviour in zebrafish across different concentrations of selenium. Warmer colours (red, yellow) represent regions where zebrafish spent more time. Cooler colours (blue) represent areas of less activity. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

= 0.002; Fig. 10). Post-hoc analysis using Dunn's multiple comparisons test confirmed a marked up-regulation of *Mn-sod* ($p < 0.02$) in larvae exposed to 50 and 100 μg/L Se compared to the control. In contrast, *Cu/Zn-sod* was significantly downregulated in the same exposure groups, as revealed by Dunnett's multiple comparisons test ($p < 0.05$) (Fig. 10). Furthermore, Se exposure also resulted in significant dysregulation of *gpx* gene expression (One-way ANOVA: $F_{3,17} = 4.97$, $p = 0.01$). Specifically, exposure to 50 and 100 μg/L Se led to significant upregulation of *gpx* expression, showing ~3-fold and 1.8-fold increases, respectively, relative to the control (Dunnett's multiple comparisons test: $p < 0.008$ for both concentrations). Moreover, although zebrafish larvae exposed to the two highest Se concentrations exhibited a relative increase in *nrf2a* expression, only the increase in the 50 μg/L exposure group was statistically significant compared to the control (Dunnett's multiple comparisons test: $p = 0.01$). In contrast, *nrf2b* expression was significantly downregulated in the 50 μg/L Se-exposure group (One-way ANOVA: $F_{3,15} = 2.94$, $p = 0.04$; Dunnett's multiple comparisons test: $p = 0.04$). Furthermore, embryonic exposure to Se significantly affected the expression of the apoptotic marker gene *caspase3* (One-way ANOVA: $F_{3,12} = 6.62$, $p = 0.007$). Zebrafish larvae exposed to 100 μg/L Se showed a significant (~1.8-fold) increase in *caspase3* transcription compared to the control (Dunnett's multiple comparisons test: $p = 0.004$; Fig. 10).

3.5.2. Expression of neural signalling and neurodevelopmental marker genes

The expression profiles of key genes involved in neural signalling and neurodevelopment in zebrafish larvae were also affected by waterborne Se exposure (Fig. 10). Notably, exposure to Se led to a significant upregulation of several genes involved in dopaminergic signalling. *MAO* expression increased significantly following Se exposure (One-way ANOVA: $F_{3,16} = 7.97$, $p = 0.002$), with post-hoc analysis showing a pronounced upregulation in the 50 μg/L Se treatment group compared to the control ($p = 0.001$). Similarly, the expression of *th1* gene also exhibited a significant increase at the 100 μg/L Se exposure ($p = 0.02$). Moreover, the expression of *robo2* ($p = 0.01$) and *sncgb* ($p = 0.03$) genes was significantly elevated in the 50 μg/L Se treatment, while *otpa* expression increased significantly at both 50 and 100 μg/L Se treatments compared to the control ($p = 0.02$).

Selenium exposure similarly affected genes involved in serotonergic

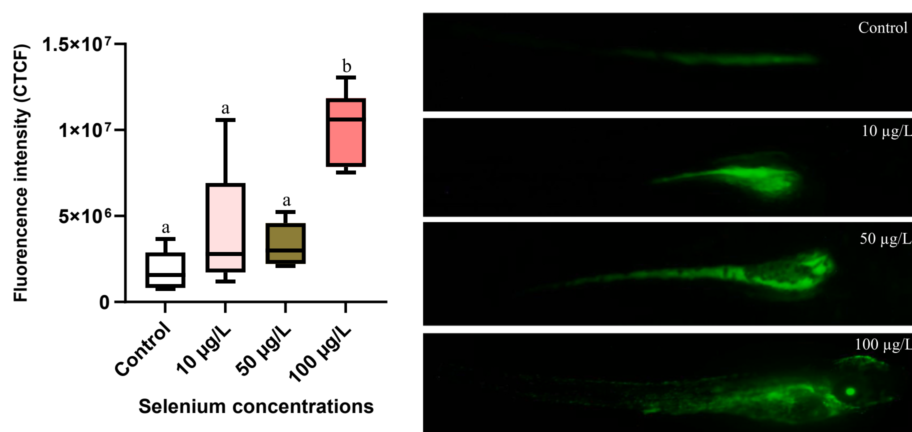

Fig. 8. Box plot indicates DCF-DA staining *in vivo* median production of ROS in 5 dpf larvae exposed to different concentrations of waterborne selenium. The horizontal center line in the boxes represents the median. The box reflects the median contained in the first and third quartiles (Q1 and Q3), with whiskers extending to minimum and maximum values. Different letters above data box denote a significant difference relative to control group at $p < 0.05$ (n = 4 replicates of 12 larvae). DCF-DA- dichloro-dihydro-fluorescein diacetate; ROS- reactive oxygen species; dpf-days post fertilization.

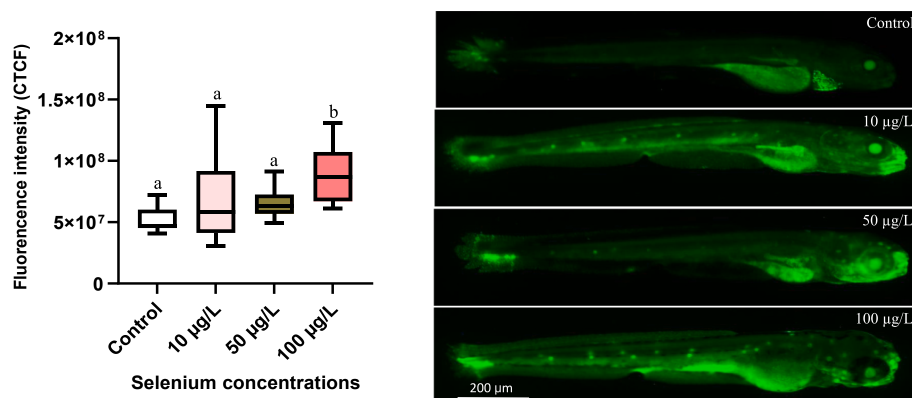

Fig. 9. Box plot indicates the effects of waterborne selenium exposure on the median production of apoptotic cells in larval zebrafish. The horizontal center line in the boxes represents the median. The box reflects the median contained in the first and third quartiles (Q1 and Q3), with whiskers extending to minimum and maximum values. Different letters above data box denote a significant difference relative to control group at $p < 0.05$ (n = 4 replicates of 16 larvae).

neurotransmission. The expression of *tph2* and *pet1* genes showed significant upregulation in the 50 μg/L Se treatment group (*tph2*: p = 0.002, *pet1*: p = 0.01), while the serotonin receptor gene *5ht2c* was significantly elevated at 100 μg/L Se exposure (p = 0.02) relative to the control. In contrast, changes in the expression of *5ht1ab* and *serta* following Se exposure were not statistically significant relative to the control ($p > 0.05$).

In addition, Se exposure also influenced the expression of genes involved in motor functions. The transcription of *isll* gene was significantly elevated in the 10 and 50 μg/L Se exposure groups, while the expression of *sox* and *insm1a* genes increased in the 50 and 100 μg/L exposure groups, respectively, relative to the corresponding controls ($p < 0.05$) (Fig. 10). Moreover, the expression of *bdnf* gene was significantly downregulated in 50 and 100 μg/L Se treatment groups compared to the control (Dunnett's multiple comparisons test: p = 0.004 and p = 0.04, respectively). However, no significant change in the expression of *huc* gene was recorded between the control and Se-treatment groups (p = 0.31) (Fig. 10).

4. Discussion

The present study demonstrated that developmental exposure to waterborne selenite can impair neurodevelopment and cause behavioural deficits in larval zebrafish. The Se exposure concentrations used in this study are comparable to the total dissolved Se levels reported in Se-contaminated natural water bodies (Kelly et al., 2018; Etim, 2017; Haque et al., 2016; Bajaj et al., 2011; Hudak, 2009). Therefore, it is plausible that fishes inhabiting such contaminated systems could suffer from Se neurotoxicity and behavioural impairments, which can ultimately affect their growth, survival, reproduction and population sustainability. It is important to note, however, that in natural aquatic ecosystems, microbial activity and interactions with sulfur compounds influence Se cycling, altering its speciation, bioavailability, and toxicity (Lenz and Lens, 2009). For example, selenite can be reduced to elemental selenium (Se^0) through reactions with sulfide and sulfite (Stolz et al., 2006) or can be biotransformed into organic Se such as Se-Met by microbes (LeBlanc and Wallschlager, 2016). Our study excluded these processes to focus exclusively on the effects of waterborne selenite on zebrafish development and behaviour under controlled laboratory conditions.

Several previous studies reported that chronic dietary exposure to organic Se (Se-Met) causes behavioural impairments in adult zebrafish by inducing oxidative stress and dysregulation of neurotransmitter systems (dopamine and serotonin) in the brain (Attaran et al., 2019, 2020; Naderi et al., 2017, 2018a). However, the present study is the first to demonstrate the neurotoxic and behavioural effects of early life stage exposure to environmentally relevant levels of dissolved inorganic Se (selenite) in larval zebrafish. Our findings suggest that the apical parameters such as hatching and survival rate, and larval deformities are not significantly affected by exposure to waterborne selenite, which is

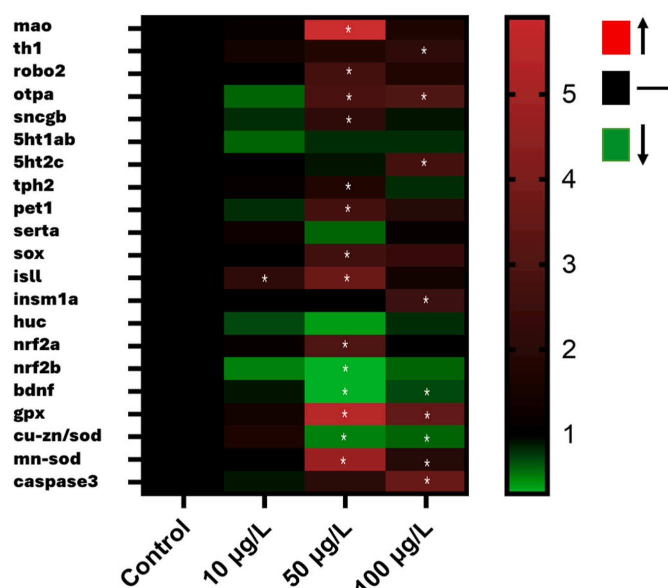

Fig. 10. Heatmap showing the relative gene expression patterns of neurodevelopmental and oxidative stress markers in zebrafish larvae post exposure to environmentally relevant concentrations of selenium at 5 dpf. Rows represent genes; columns, selenium exposure concentrations; black bars represent baseline (control group). Red and green bars represent an upregulation and downregulation of genes, respectively. The intensity of the colour increases as the expression differences increase as shown in the bar at the right. Asterisk indicates that data are significantly different from the control group. Values are presented as mean ± SEM, there were five replicates, and each replicate contained 50 larvae. *p < 0.05. dpf-days post fertilization. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

consistent with previous studies with dissolved inorganic Se (selenite and selenate) toxicity in zebrafish (Hariharan et al., 2024; Arnold et al., 2016). However, we observed that the behavioural responses, including thigmotaxis, reflexive response to light and dark, social preference and object recognition response, are significantly impaired in larval zebrafish following exposure to waterborne selenite, particularly at Se exposure levels ≥50 μg/L.

Behaviour is a highly structured process regulated by complex neural networks that are essential for the fitness and survival of organisms (Kane et al., 2005). In ecotoxicology, behavioural alterations serve as sensitive and valuable endpoints for detecting neurological impairments caused by pollutants (Tierney, 2011; Kane et al., 2005). Previous studies have demonstrated that neurotoxicity induced by environmental contaminants is closely associated with behavioural changes during the early developmental stages of zebrafish (Chen et al., 2012a, 2012b). Given the sensitivity of the nervous system to exogenous toxicants, particularly during embryonic neurogenesis, behavioural assays have proven to be a reliable and effective method for assessing chemical exposure in the early life stages of fish (Wang et al., 2019; Sloman and McNeil, 2012). Our study further corroborates this growing body of evidence by indicating that behaviours are likely more sensitive indicators of the developmental toxicity of the most toxic form of inorganic Se, selenite, in fishes.

In the current study, we observed significant disruptions in locomotor behaviour in larval zebrafish exposed to elevated Se concentrations, particularly under consecutive light and dark conditions. The zebrafish larvae exposed to the highest levels of Se (50 and 100 μg/L) displayed a marked reduction in median swimming speed, total distance travelled, and the number of transitions between light and dark phases. These behavioural alterations are consistent with the behavioural toxicity of other trace metals, such as copper and silver, in larval zebrafish (Zhao et al., 2019; Zhang et al., 2015). Additionally, in the present study, Se-exposed larvae exhibited impaired thigmotactic behaviour, as indicated by their reduced exploration of the outer zone and increased time spent in the inner zone of the exposure chamber. Furthermore, we observed a reduction in transition numbers, which measures the absolute turn angle and is a sensitive marker of motor coordination, indicating that the larvae experienced disorganized locomotion (Bortolotto et al., 2014; Blazina et al., 2013). These results suggest elevated Se exposure induces hypoactivity and a disordered swimming pattern in zebrafish larvae. Our observations are in agreement with Hariharan et al. (2024), who also reported impaired anxiety-like behaviours such as light and dark preference behaviour and exploratory diving response in larval zebrafish (6 dpf) following exposure to waterborne selenite, although at a Se concentration range (100–275 μg/L) much higher than that used in the present study.

Motor neuron and dopaminergic neuron health are essential for maintaining normal locomotor behaviour, and disruptions to these neurons are linked to neurodegenerative diseases (Karam et al., 2010; Singer et al., 2007; Rowland and Shneider, 2001). In zebrafish, genes such as *sox*, *isl1*, and *insm1a* play key roles in motor neuron differentiation and development. Reduced expression of these genes, as observed in Se-treated zebrafish larvae in our study, suggests that Se impairs motor neuron differentiation and function, contributing to the observed motor dysfunction. Previous studies have shown that the loss of *sox* and *isl1* disrupts motor neuron axonal pathfinding, while deficiency in *insm1a* leads to defective motor neuron differentiation (Xing et al., 2021; Gong et al., 2017). In addition to motor neurons, Se can also affect dopaminergic neurons, which are crucial for regulating spontaneous motor activity. Our results showed an upregulation of key dopaminergic genes (*mao*, *th1*, *otpa*, *sncgb*) in zebrafish exposed to high Se levels. The gene *mao* encodes the mitochondrial enzyme monoamine oxidase that catalyzes the breakdown of neurotransmitters like dopamine, serotonin, and norepinephrine, while the *bdnf* gene plays an important role in neural development. Increased activity of this enzyme enhances mitochondrial ROS production (Santin et al., 2021), thus the upregulation of *mao* in Se-exposed larvae suggests elevated oxidative stress. Furthermore, *otpa* contributes to the specification of *th1* expressing dopaminergic neurons (Fernandes et al., 2013). Thus, upregulation of *th1*, which encodes tyrosine hydroxylase (a critical enzyme for dopamine synthesis), further supports the hypothesis that Se exposure increases dopamine levels, leading to impaired motor activity. Dysregulation of *mao*, *robo2*, *otpa*, and *bdnf* has been linked to disrupted dopaminergic signalling, abnormal neural development, and behavioural impairments such as reduced locomotion and anxiety-like behaviours in zebrafish (Xia et al., 2021; Naderi et al., 2018a; Wircer et al., 2017).

Both social behaviour and exploratory behaviours are vital to fishes, playing key roles in foraging, reproduction, and predator avoidance. These behaviours depend on a well-functioning nervous system but can be disrupted by exposure to contaminants. In the present study, we observed a significant reduction in social preference and exploratory behaviours in zebrafish larvae exposed to increasing concentrations of Se. It is to be noted here that in the present study, the behavioural responses in larval fish in Se treatments were not influenced by their apparent poor health, since we only used fish that demonstrated no apparent symptoms of poor health (e.g., erratic swimming, freezing, or jumping) and actively explored the maze during the training sessions. Previous research has demonstrated that chronic dietary exposure to Se-Met impairs social and antipredator behaviours in adult zebrafish by affecting serotonergic neurotransmission in the brain (Attaran et al., 2019). Serotonin (5-HT) is a critical neurotransmitter in the central nervous system (CNS), influencing a wide array of functions, including mood regulation, social responses, and cognitive processes (Canli and Lesch, 2007). The serotonergic system operates through multiple interconnected processes, including serotonin synthesis, release, reuptake, degradation, and receptor activity (Sahu et al., 2018). In this study, zebrafish larvae exposed to waterborne selenite exhibited upregulation of key genes involved in serotonin regulation, including *tph2*, *pet1*, and

5ht2c, which are critical for serotonin synthesis and reuptake. These findings are in agreement with the findings of Attaran et al. (2019), who reported that exposure to Se-Met for 60 days increased the mRNA expression of *tph2* and *serta* in the brain of adult zebrafish. The significant upregulation of *tph2*, *pet1* and *5ht2c* observed in the highest Se exposure groups in the present study suggests potential disruptions in serotonergic neurotransmission, which could have mediated the observed behavioural impairments, including reduced locomotor activity, increased anxiety, and diminished exploration (Herculano and Maximino, 2014; Nordquist and Oreland, 2010). It is also possible that impaired locomotor activity, another consequence of selenite exposure, might have also contributed to the disruption of normal social behaviours. Nevertheless, these behavioural impairments may elevate chronic stress and risks to predation, ultimately leading to negative ecological consequences that impact growth, reproduction, and overall population dynamics (Rice and Barone, 2000).

Exposure to both organic (SeMet) and inorganic (selenite) forms of Se increases intracellular ROS production and thereby induce oxidative stress, which is a key driver of Se toxicity in fishes (Misra et al., 2010). The present study revealed a dose-dependent increase in DCF-DA fluorescence intensity in Se-treated zebrafish larvae, indicating increased ROS accumulation and oxidative stress. Elevated oxidative stress has been linked to various neurodegenerative diseases and behavioural impairments in both humans and animals (Glade, 2010). Specifically, in zebrafish, oxidative stress in the brain has been shown to impair associative and spatial learning (Ruhl et al., 2016), while SeMet-induced oxidative stress in the brain has been found to be associated with the disruption of latent learning, anti-predator, and social behaviours in zebrafish (Attaran et al., 2019, 2021; Naderi et al., 2017, 2018a). Moreover, increased oxidative stress has been implicated in both depression-like behaviours and cognitive deficits in other animal models, such as rats (Patki et al., 2013). Therefore, it is logical to suggest that oxidative stress played a key role in mediating the neurodevelopmental and behavioural deficits observed in zebrafish larvae in our study.

Contaminant-induced oxidative stress plays a crucial role in mediating developmental neurotoxicity in animals (Sayre et al., 2008). Early life stages of fishes are particularly vulnerable to oxidative stress (Felix et al., 2016), which results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses. In our study, zebrafish larvae exposed to higher Se concentrations exhibited dysregulation in the expression of key antioxidant genes, including *gpx1*, *Cu/Zn-sod* and *Mn-sod*, which encode enzymes that represent the first line of defense against oxidative damage (Ighodaro and Akinloye, 2019). Specifically, superoxide dismutase (*SOD*) converts superoxide radicals into hydrogen peroxide, which is then neutralized by glutathione peroxidase (*GPx*) into water and oxygen. In the present study, we also observed changes in the expression of *Nrf2* gene, a key transcription factor responsible for activating the antioxidant response by binding to the antioxidant response element (ARE) in gene promoters (Ma, 2013). Our findings revealed an initial increase in the *sod* expression in zebrafish larvae at 10 μg/L Se treatment followed by a decrease in 50 and 100 μg/L Se treatments. This pattern suggests that while oxidative stress was initially mitigated through the activation of antioxidant enzymes, exposure to elevated Se levels likely resulted in increased ROS production which overwhelmed the cellular antioxidant capacity. Nevertheless, *GPx* expression increased consistently across all the Se-treatment groups, indicating a sustained antioxidant response. These observations align with previous studies that reported induction of oxidative stress and dysregulation of antioxidant systems, including *Nrf2*, *SOD*, and *GPx*, in fish exposed to Se (Naderi et al., 2018b; Jiang et al., 2014; Misra et al., 2012; Misra and Niyogi, 2009; Liu et al., 2006).

Oxidative stress has been suggested to induce apoptotic signalling pathways leading to cell death (Chen et al., 2008). Our results are in agreement with this observation as Se-exposed zebrafish larvae exhibited upregulation of *caspase3* gene, a key marker of cellular apoptosis. Moreover, we also observed increased AO fluorescence in zebrafish larvae following exposure to Se, further indicating that selenite exposure induced apoptotic cell death. Hariharan et al. (2024) also reported a significantly higher number of apoptotic cells in zebrafish larvae exposed to elevated waterborne selenite. A balance between cell proliferation and apoptosis is essential during embryogenesis, particularly in processes such as neural tube development and trunk and tail elongation (Ross, 1996). In the present study, we observed abnormal patterns of proliferation and apoptosis in Se-exposed larvae, which likely contributed to the observed neurodevelopmental impairments. Apoptosis is an energy-dependent process and requires ATP for the activation of caspases (Elmore, 2007). Therefore, disruptions in cellular energy metabolism could impair normal development, leading to reduced growth and physiological impairments in developing organisms (Du et al., 2018; Souders et al., 2018; Pinho et al., 2013). Our findings showed a significant reduction in both body length and weight of zebrafish larvae exposed to Se at concentrations of 50 and 100 μg/L at 30 dpf, which may be attributed to altered energy metabolism. This reduction in growth was likely accompanied by a decrease in metabolic rate and hypoactivity, as organisms may reduce activity to conserve energy when cellular energy production is compromised (Jimenez et al., 2014; Kramer, 1987).

AO staining results in the present study indicated that Se exposure increased neuronal apoptosis, particularly in regions of the brain such as the ventral telencephalon and mesencephalon optic parietal regions. Apoptosis appeared symmetrically distributed and increased in severity with higher Se concentrations. It is therefore reasonable to suggest that the neuronal apoptosis likely contributed to the observed behavioural deficits, such as spontaneous motion inhibition in zebrafish larvae.

5. Conclusions

Overall, our study provides critical insights into the neurodevelopmental and behavioural implications of developmental exposure to environmentally relevant concentrations of waterborne Se (as selenite) in larval zebrafish. We demonstrated that waterborne selenite induces cellular ROS production, leading to neuronal apoptosis and dysregulations of genes involved in neural development and signaling pathways. The molecular disruptions subsequently lead to behavioural and physiological impairments, ultimately reducing the overall fitness (e.g., growth) and performance (e.g., swimming, stress response, social and exploratory behaviours) of developing zebrafish. Normal health and wellbeing of larvae are crucial for maintaining healthy fish populations, and the findings of our study enhance our understanding of the adverse impacts of selenite in sensitive early life stages of fishes, which may help in risk assessment of Se contamination in aquatic ecosystems. It is important to note though that fish behaviour is a highly complex process and is regulated by the interactions of multiple neurotransmission systems in the brain. A key limitation of our study was that the selenite-induced neurodevelopmental alterations were evaluated in whole zebrafish larvae rather than in the brain. Thus, future studies should focus on examining how Se influences the neural signaling pathways in specific brain regions (e.g., telencephalon), which regulate complex behaviours, to acquire a more precise and in-depth understanding of Se neurotoxicity in fishes.

CRediT authorship contribution statement

Md Helal Uddin: Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jinnath Rehana Ritu:** Writing – review & editing, Investigation. **Douglas P. Chivers:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition. **Som Niyogi:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2025.121240.

Data availability

Data will be made available on request.

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Volume 274, Issue , 1 June 2025, Page

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Environmental Research 274 (2025) 121463

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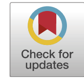

Corrigendum to 'Neurodevelopmental and behavioural effects of waterborne selenite in larval zebrafish (Danio rerio)' [Environ. Res. 273 (2025) 121240]

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ABSTRACT

Selenium (Se) is an essential element that becomes highly toxic to fish at elevated exposure levels. Although the neuro-behavioural effects of organic Se are well documented in adult fish, the effects of inorganic Se (selenite) on neurodevelopment and behaviour, particularly in early life stages, remain poorly understood. In this study, zebrafish embryos were exposed to different environmentally relevant concentrations of waterborne Se (0 (control), 10, 50, 100 μg/L; as selenite) from 4 hours post-fertilization to 30 days post-fertilization. We evaluated neurodevelopmental and behavioural outcomes, along with oxidative stress as a potential mechanism of selenite neurotoxicity. Fish larvae exposed to higher Se concentrations (50 and 100 μg/L) exhibited significant behavioural impairments, including reduced thigmotaxis and reflexive movement, spent significantly less time (60 %) near their conspecifics, and lower exploratory response (1.5 fold) to the novel object. These behavioural deficits were associated with elevated oxidative stress, as indicated by increased (5.4 fold) DCF-DA fluorescence intensity and dysregulation (0.6–6.4 fold change) of key antioxidant genes. Additionally, selenite exposure led to increased apoptotic cell death ($p < 0.001$), and reduced length (16 %) and weight (33–47 %) of zebrafish larvae in 50 and 100 μg/L Se exposure groups compared to the control group. Neurodevelopmental disruptions were evident through altered expression of dopaminergic (*mao, th1, otpa*; all $p < 0.05$) and serotonergic (*tph2, pet1, 5ht2c*; all $p < 0.05$) pathway genes, critical regulators of behaviour in fishes. Overall, our findings suggest that selenite-induced oxidative stress and neurodevelopmental gene dysregulation contribute to the observed behavioural impairments in developing zebrafish, highlighting the potential risks of Se exposure during early life stages.

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