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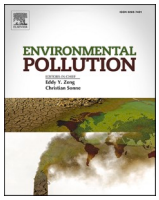

Oxidative stress-mediated neurotoxicity of para-xylene and neuroprotection by gluconolactone in *Xenopus laevis*☆

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ABSTRACT

Para-xylene (PX) is a commonly used industrial solvent that poses significant neurotoxic risks, however, the underlying physiological mechanisms remain inadequately defined. In this study, we exposed *Xenopus laevis* tadpoles to PX and used RNA sequencing and enzymatic assays to explore the underlying neurotoxic mechanisms. Our findings revealed a marked reduction in the expression of genes associated with oxidative stress defense, notably superoxide dismutase (*sod1.L*) and catalase (*cat.L*). These observations were validated by quantitative PCR and enzyme activity assays, which confirmed decreased SOD and CAT activities alongside elevated levels of oxidative damage markers, including malondialdehyde (MDA) and lactate dehydrogenase (LDH). PX treatment leads to increased neuronal apoptosis and abnormal swimming behavior. Notably, the co-administration of glucuronolactone (GA) with PX restored the activities of these critical enzymes and reduced oxidative damage, suggesting a mitigating effect of GA on PX-induced stress. Further validation using diethyldithiocarbamate (DDC) to inhibit SOD activity underscored the enzyme's pivotal role in mediating PX toxicity. Additionally, TUNEL assays demonstrated that GA effectively prevented neuronal apoptosis in the optic tectum, while behavioral assessments indicated a recovery in normal swimming patterns. Collectively, these results indicate that PX exposure triggers oxidative stress, leading to neuronal damage and behavioral deficits, whereas GA confers a protective effect by re-establishing antioxidant balance and reducing cell death.

1. Introduction

Industrial activities such as combustion, vehicular emissions, and petroleum processing release significant amounts of aromatic hydrocarbons, including benzene, toluene, ethylbenzene, and xylene (BTEX) (Alexopoulos et al., 2006). These volatile and fat-soluble chemicals are widely used in industry but pose serious environmental and occupational health risks (Georgieva et al., 2002; Ghahri et al., 2024; Laine et al., 1993; Uzma et al., 2010). Mobile BTEX fractions can volatilize into surface water or percolate into groundwater, allowing these compounds to enter aquatic ecosystems through leaching, runoff, or effluent discharge (ATSDR, 2007). Their ability to easily cross biological membranes leads to accumulation in fatty tissues, particularly in the central nervous system (CNS), potentially contributing to neurological, cancerous, and blood-related disorders (Bahadar et al., 2014; Gralewicz et al., 1995; McKenzie et al., 2012). Notably, xylene has been recognized as a priority pollutant, present at nearly half of the toxic waste sites identified by the United States Environmental Protection Agency (EPA) (Adebambo et al., 2020). Environmental concentrations of xylene vary depending on local conditions such as industrial emissions and atmospheric dispersion (ATSDR, 2007). Exposure to xylene, even at relatively low concentrations (e.g. 50 ppm) via inhalation, has been linked to symptoms such as drowsiness, fatigue, and headaches, while prolonged exposure is associated with cognitive decline and neurodegenerative conditions (Alexopoulos et al., 2006; Bahadar et al., 2014; Langman, 1994; Niaz et al., 2015).

The toxic effects of BTEX compounds, and xylene in particular, arise from several mechanisms including disruptions in neurotransmitter function, neural inflammation, oxidative stress, and resulting behavioral impairments (Andersson et al., 1981; Bahadar et al., 2014; Wang et al., 2020; Zhou et al., 2024). Researchers have used diverse experimental models from cell cultures and insects to rodents to explore

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xylene-induced neurotoxicity (Adebambo et al., 2020; Al-Ghamdi et al., 2004; Bushnell, 1988; Gagnaire et al., 2007; Liu et al., 2010; Neuparth et al., 2014; Padilla et al., 1992; Pajaro-Castro et al., 2019; Rosen et al., 1986; Selgrade et al., 1993; Singh et al., 2009). Unlike terrestrial organisms that are primarily exposed to volatile chemicals via inhalation or dermal absorption, *Xenopus laevis* (*X. laevis)* tadpoles inhabit fully aquatic environments and absorb contaminants directly from the surrounding water through their skin and gastrointestinal tract. Among aquatic models, *X. laevis* has proven especially useful for studying the effects of environmental toxicants on neural development and function (Beck and Slack, 2001; Buryskova et al., 2006; Gao and Shen, 2021; Pratt and Khakhalin, 2013). Our previous work demonstrated that exposure to *para*-xylene (PX) triggers neuronal cell death and disrupts both structural and synaptic plasticity in the developing *X. laevis* brain. Notably, cotreatment with glucuronlactone (GA) reduced these negative outcomes (Gao et al., 2016; Liao et al., 2020). Despite these findings, the exact neuroprotective mechanisms of GA remain unclear, and there is a need for a comprehensive analysis of gene expression changes following PX exposure. In this study, we use transcriptome sequencing to identify differentially expressed genes and molecular pathways involved in pollutant-induced toxicity within the optic tectum of *X. laevis* (Chen et al., 2024a; Liao et al., 2022; Sai et al., 2019).

Oxidative stress plays a central role in solvent-induced toxicity, resulting from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses (Saeidnia and Abdollahi, 2013). Under physiological conditions, cells maintain a careful balance between oxidative and antioxidative processes. However, exposure to aromatic hydrocarbons disrupts this balance, leading to excessive ROS production and subsequent cellular damage (Xia et al., 2017; Zhou et al., 2024). Biomarkers such as superoxide dismutase (SOD), glutathione reductase (GSR), catalase (CAT), malondialdehyde (MDA), and lactate dehydrogenase (LDH) are commonly used to assess antioxidant capacity, lipid peroxidation, and cell membrane integrity. Studies in workers exposed to BTEX compounds have consistently shown increased ROS production, which correlates with various pathological conditions (Georgieva et al., 2002; Ghahri et al., 2024; Liu et al., 2024; Rizk et al., 2020; Uzma et al., 2010; Wang et al., 2020; Xia et al., 2017; Xiong et al., 2016; Zhou et al., 2024). However, findings on changes in SOD activity following xylene exposure have been inconsistent across different models (Costa et al., 2006; Kum et al., 2007; Neuparth et al., 2014; Pajaro-Castro et al., 2019), leaving the specific role of oxidative stress in xylene-induced neurotoxicity and its underlying molecular mechanisms insufficiently defined.

To address these questions, our study investigates the impact of PX exposure on global gene expression and oxidative stress pathways in the developing *X. laevis* brain. We integrate RNA sequencing with biochemical assays that evaluate antioxidant enzyme activities and markers of oxidative damage to elucidate the molecular basis of PX-induced neurotoxicity. Additionally, we examine the potential neuroprotective effects of GA by assessing its influence on oxidative stress-mediated apoptotic pathways in the optic tectum and its contribution to the recovery of animal behavior. Our findings aim to provide deeper insight into the molecular mechanisms underlying PX neurotoxicity and enhance the broader understanding of how environmental pollutants affect neural development and behavior.

2. Methods

2.1. Animals

Adult *X. laevis* frogs were maintained under standard laboratory conditions (19–22 °C, 12 h light/12 h dark cycle) using a standardized *Xenopus* breeding system (LY-ZC-9, China). Albino or wild type embryos were obtained by injecting human chorionic gonadotropin (HCG) for females (400 IU) and males (200 IU) in the system. Fertilized eggs were maintained in dechlorinated, aerated aquaria with partial daily water changes to preserve water quality. Upon hatching, embryos were transferred to 0.1 × Steinberg's solution (in mM: 10 HEPES, 58 NaCl, 0.67 KCl, 0.34 $Ca(NO_3)_2$, 0.83 $MgSO_4$, pH 7.2) and incubated at 22 °C. Tadpoles were not fed during the exposure period and were monitored daily for general health and survival. Developmental stages were determined according to Nieuwkoop and Faber's developmental table to ensure consistency across experimental groups (Zahn et al., 2022). All animal procedures were approved by the Animal Ethics Committee of Hangzhou Normal University (permit number: 2023049). Animals were anesthetized in 0.02 % MS-222 (Tricane methanesulfonate, Sigma-Aldrich) for experimental manipulations.

2.2. Chemicals and treatment

Para-xylene (PX; CAS number: 106-42-3; purity: >99 %; Sangon Biotech) was stored in a light-protected brown bottle and freshly diluted in Steinberg's solution prior to use. The exposure concentration of PX (1 mM, approximately 106 ppm) and duration (72 h) was selected based on relevance to environmental and occupational exposure levels, as established in previous studies (Laine et al., 1993; Langman, 1994; Liao et al., 2020). D-(+)-Glucuronolactone (GA; CAS number: 32449-92-6; purity: 99 %; Aladdin) was applied at 5.7 mM, as established in prior studies (Gao et al., 2016; Liao et al., 2020; Yu et al., 2018). Diethyldithiocarbamate (DDC; CAS number: 148-18-5; purity: >99 %; Selleck), a specific Cu/Zn-SOD inhibitor, was used at 50 nM to block SOD activity (Dumay et al., 2006).

Stage 46 *X.laevis* tadpoles were randomly assigned to experimental groups and housed in 500 mL glass bowls containing 10 individuals per bowl, filled with 0.1 × Steinberg's solution under a 12 h light/12 h dark cycle at 22 °C. Control and treatment groups were maintained under identical environmental conditions. The exposure medium was renewed daily to ensure chemical stability and minimize waste accumulation. Tadpoles were monitored daily, and any dead or unhealthy individuals were promptly removed. All procedures followed the guidelines outlined in OECD Test Guideline 203 (Fish, Acute Toxicity Test) (OECD, 2019) to ensure standardized toxicity assessment conditions.

2.3. RNA extraction and sequencing

Optic tecta from treated and control groups (40 brains each group) were carefully dissected under a stereomicroscope (Nikon SMZ-1500) and immediately snap-frozen in liquid nitrogen. Total RNA was extracted using the AxyPrep™ RNA Miniprep Kit following the manufacturer's protocol. RNA integrity was assessed using an Agilent 2100 Bioanalyzer, with samples having RNA integrity numbers (RIN) > 7.0 considered suitable for sequencing. RNA-seq libraries were constructed using Illumina TruSeq RNA Sample Prep Kit and sequenced on an Illumina NovaSeq platform with 150 bp paired-end reads. Following quality assurance, the filtered clean reads were aligned to the *X. laevis* v10.1 genome available via Xenbase (http://www.xenbase.org). Differentially expressed genes (DEGs) were analyzed using DESeq2 software, with a false discovery rate (FDR) *P*-value <0.05 and a log2 fold change >1. Functional annotation analysis was carried out using the DAVID bioinformatics resource (https://david.ncifcrf.gov).

2.4. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was extracted from 30 optic tecta using the Multisource Total RNA Miniprep Kit (Axygen). cDNA was reverse-transcribed from RNA using PrimeScript RT reagent Kit (TaKaRa) and quantitated with UltraSYBR Mixture (Cwbio). RNA quality and purity were verified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). Samples were analyzed using a PCR system (CFX 96, Bio-Rad). Thermal cycling was performed for 39 cycles; each cycle consisted of 15 s of denaturation at 95 °C, with 60 s at each annealing temperature of 60 °C.

Expression values of three replicates was normalized to GAPDH and analyzed using the 2–ΔΔCt approach. The primers (Table 1) used for the target genes were designed using Primer Premier 5.0 software, and synthesized by Sangon Biotech (Shanghai, China).

2.5. TUNEL assay

Tadpoles were fixed in 4 % paraformaldehyde (PFA) overnight at 4 °C. The brains were dehydrated in 30 % sucrose for 48 h and embedded in optimal cutting temperature (OCT) compound. The samples were sectioned and washed with 0.1 M PB. Permeabilization is performed with 0.3 % Triton X-100, then sections were incubated with the TUNEL reaction mixture at 37 °C for 60 min in the dark according to the manufacture directions (C1089, Beyotime, China). After TUNEL staining, the brain sections were counterstained with DAPI (1:10,000) for 5 min to visualize cell nuclei. All sections were mounted and scanned using a confocal microscope (LSM710, Zeiss, Germany). A total of 4–5 tadpoles per group were used for the TUNEL assay. The TUNEL-positive apoptotic cells across all sections were counted using an image processing software (iMaris, Switzerland).

2.6. Enzyme activity assays

The optic tecta were dissected and homogenized in 500 μL of 0.65 % NaCl solution. Enzyme activities were determined using commercially available assay kits from Nanjing Jiancheng Bioengineering Institute (SOD: A001-3-2; CAT: A007-1-1; MDA: A003-1-2; LDH: A020-2-2). Protein concentrations were measured by BCA assay using a Nanodrop (Thermo Scientific, 2000c; Waltham, USA). SOD and CAT activities were measured spectrophotometrically at 450 nm and 405 nm, respectively, while MDA activity was quantified based on thiobarbituric acid reactive substances (TBARS) levels and LDH activity was evaluated by monitoring NADH consumption. The results are expressed as U/mg protein for SOD and CAT, nmol/mg protein for MDA, and U/L for LDH.

2.7. Behavioral analysis

Stage 46 tadpoles were randomly assigned to treatment groups and incubated in 500 mL glass containers, with 10 tadpoles per container and 16 tadpoles per treatment group. The treatments included 1 mM PX, 50 nM DDC, or 5.7 mM GA, all prepared in 0.1 × Steinberg's solution. Control tadpoles received only the vehicle solution. The medium was replaced daily, and the tadpoles were incubated for 72 h at 22 °C. The 72-h exposure period was selected based on our study, which showed that significant alterations in oxidative enzyme activity and neuronal apoptosis occur within this time frame. Tadpole swimming behavior was conducted between 1 p.m. and 3 p.m. using the Daniovision system (Noldus, Wageningen, Netherlands) under uniform lighting conditions. Recordings were captured at a resolution of 1024 × 768 pixels and 25 frames per second (fps). One-minute video recordings were analyzed with EthoVision XT11 software to quantify behavioral patterns according to our previous studies (Gao et al., 2022).

2.8. Statistics

All data were analyzed using GraphPad prism software (version 10.1.2). For comparison between two groups, a two-tailed Student's t-test was used. For comparisons among multiple groups, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed. Data normality was assessed using the D'Agostino and Pearson omnibus normality test. All measurement data were represented as mean ± SEM. A P-value <0.05 was considered statistically significant. Experiments and analysis were performed blind to the experimental condition unless otherwise noted.

3. Results

3.1. RNA sequencing identifies oxidative stress-related gene changes

To further explore how PX alters gene expression, we exposed stage 46 tadpoles with 1 mM PX for 72 h and collected their optic tecta for RNA sequencing. The results revealed that the PX exposure induced dramatic alterations in the global gene transcription profiles. Our analysis identified and annotated 37,409 genes expressed in the optic tectum of tetraploid *X. laevis*, including two sets of homologous gene (labeled L for longer and S for shorter versions). By comparing gene expression levels between PX-treated and control groups, we found 697 genes (1.8 %) were up-regulated and 2981 genes (7.9 %) were down-regulated in PX-treated optic tecta (Fig. 1A, Supplementary Table 1).

Pathway analysis using KEGG mapping indicated that PX exposure strongly affected several signaling pathways associated with oxidative stress, including efferocytosis (xla04148), the MAPK signaling pathway (xla04010) and the FoxO signaling pathway (xla04068) (Fig. 1B–Supplementary Table 2). Gene ontology (GO) analysis with false discovery rate (FDR) P-value <0.05 further highlighted significant changes in biological processes such as cell communication (GO:0007154), signal transduction (GO:0007165), regulation of biological process (GO:0050789) and cellular response to stimulus (GO:0051716). Molecular function analysis indicated alterations in genes associated with small molecule binding (GO:0036094), enzyme regulator activity (GO:0030234), transcription coregulator activity (GO:0003712), and protein-macromolecule adaptor activity (GO:0030674) (Fig. 1C) (Supplementary Table 3). The affected pathways (MAPK, FoxO), processes (efferocytosis, signal transduction), and the molecular changes are central to managing oxidative stress, inflammation, and cell survival or death. These findings suggest that PX induces brain toxicity by altering oxidative stress-related gene expression.

Further analysis revealed that several key oxidative stress-regulating genes were differentially expressed. Specifically, genes encoding superoxide dismutase (*sod1.L*), glutathione reductase (*gsr.L*) and catalase (*cat.L*), were downregulated whereas glutathione peroxidase 3 (*GPx3.L*) was upregulated. To validate the reliability of the transcriptome sequencing data, we analyzed these DEGs using qRT-PCR with GAPDH as an internal control (see Table 1 for primer sequences). The qRT-PCR

Table 1
Primers used for qRT-PCR analysis.

Target gene	Primer	Nucleotide sequence (5'→3')	Amplicon Size (bp)	Sequence ID
cat.L	Forward	GCAGACACCCAGATGAACCA	169	NM_001095194.1
	Reverse	AGTGTGCCATCTCGTCAGTG		
sod1.L	Forward	ATGGGGACAGTTGTGTCACG	230	NM_001372113.1
	Reverse	ATATGGAAGCCGTGCAGTCC		
gpx3.L	Forward	TGTGGACCAGAAATCGGTGG	119	NM_001091850.1
	Reverse	AGGTACTTCCCTTGGTAGGCT		
gsr.L	Forward	CTCTACCAACAAGCCTGGGG	199	NM_001095853.1
	Reverse	GAGCAGTGGCTTCTGTCGAA		
gapdh.S	Forward	CCCCTCCAGCATTAAGTGGG	121	NM_001087098.1
	Reverse	AGATAACGACACGCTTGGCA		

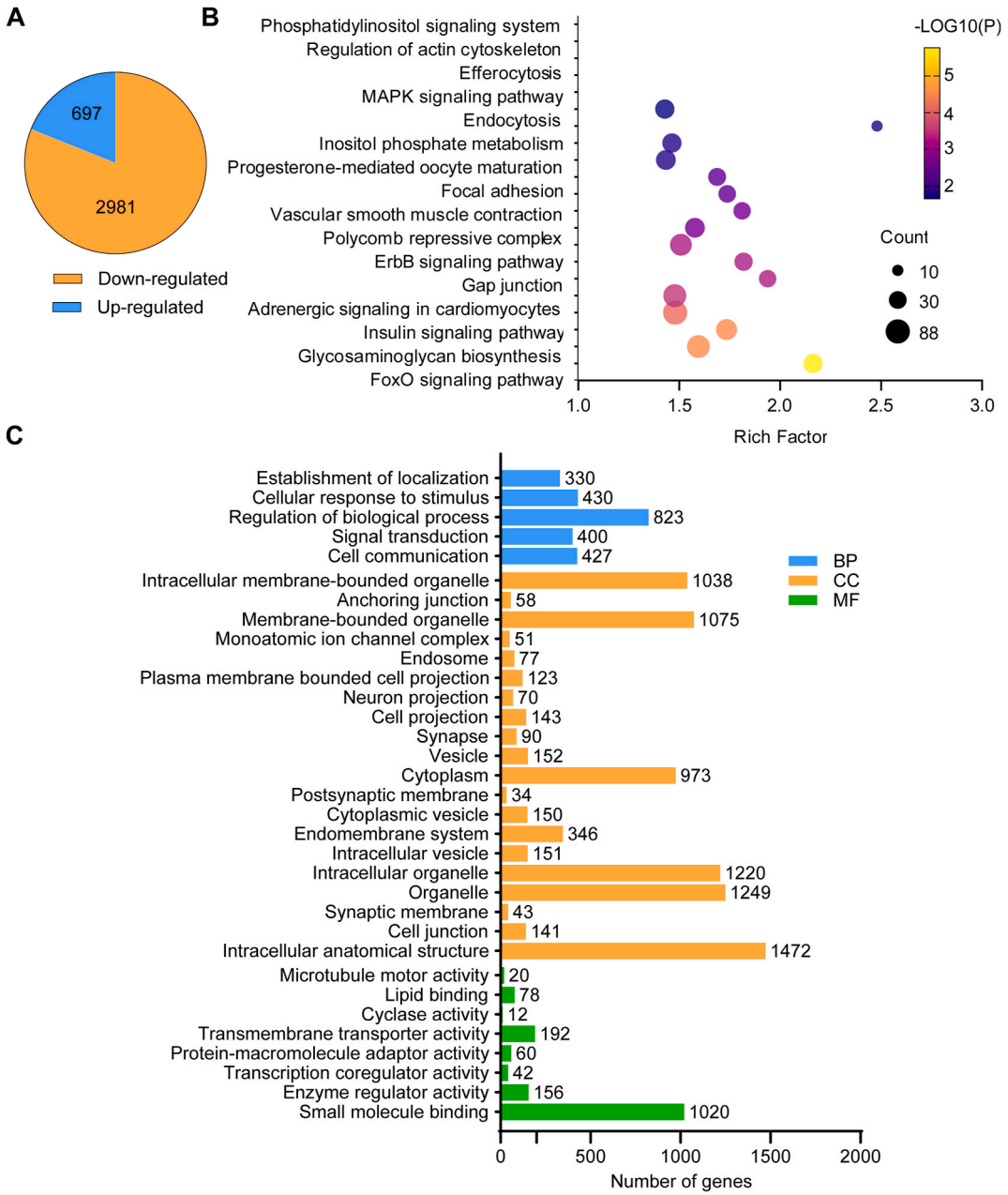

Fig. 1. Transcriptomic analysis in PX-treated optic tectum. (A) Pie chart, showing the distribution of up- and down-regulated genes in PX-exposed brains. (B) The bubble diagram of KEGG pathway analysis, highlighting differentially expressed genes (DEGs) with FDR *P*-value <0.05; the size of each bubble represents the number of enriched genes. (C) Gene ontology categories pattern of the down-regulated genes in PX-treated group. Distribution of the GO categories were assigned into three categories: cellular components, molecular functions, and biological processes.

analysis confirmed the RNA-seq findings, showing consistent down-regulation of *sod1.L*, *gsr.L*, and *cat.L*, along with an increase in *GPx3.L* expression in PX-treated samples (Fig. 2A–D). These findings support the notion that PX treatment reduces the brain's ability to counteract oxidative stress. The upregulation of *GPx3.L* may represent a compensatory response to increased oxidative stress levels.

3.2. GA restores enzyme activity and reduces oxidative damage

To directly evaluate the oxidative activities induced by PX treatment, we compared the enzyme activities among the groups. We found that the activities of SOD and CAT were significantly reduced in PX-treated 72 h tadpoles (Fig. 3A–B). Co-administration of PX and GA for 72 h effectively mitigated the enzymatic disruptions caused by PX exposure (Fig. 3A–B). To further demonstrate the PX-induced oxidative stress and cell damage, we measured the MDA and LDH activities. Our results indicated that both MDA and LDH activities were significantly elevated in response to PX treatment, whereas co-application with GA partially rescued the toxic effects of PX (Fig. 3C–D). These findings suggested that PX-induced oxidative stress contributes to brain toxicity, which can be alleviated by GA treatment.

3.3. PX-induced toxicity is mediated by SOD signaling

To clarify the role of SOD in PX-induced toxicity, we used DDC to incubate the tadpoles for 72 h. Our results revealed that DDC exposure led to a significant reduction in SOD enzyme activity, which can be reversed by GA co-treatment (Fig. 4A). Interestingly, DDC also reduced

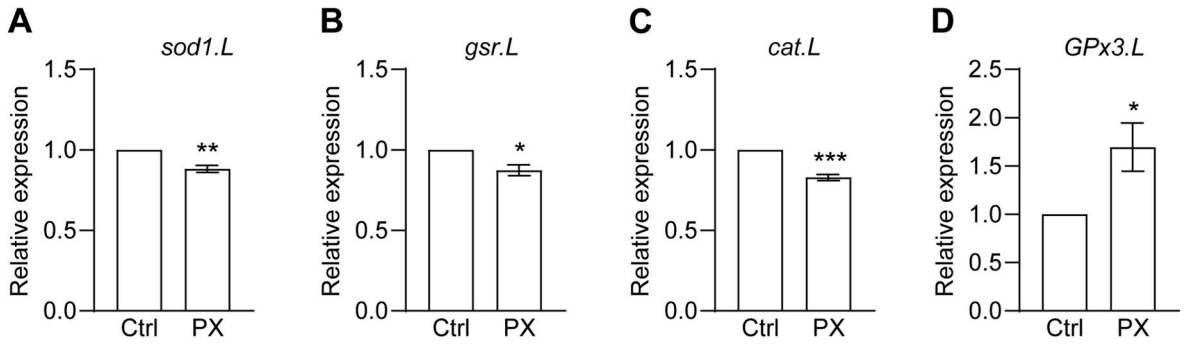

Fig. 2. Quantitative qRT-PCR validation of core genes identified from RNA-Seq analysis. (A–D) Real-time PCR data assessing the expression levels of *sod1.L* (A), *gsr.L* (B), *cat.L* (C) and *GPx3* (D), with each condition comprising three biological replicates. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

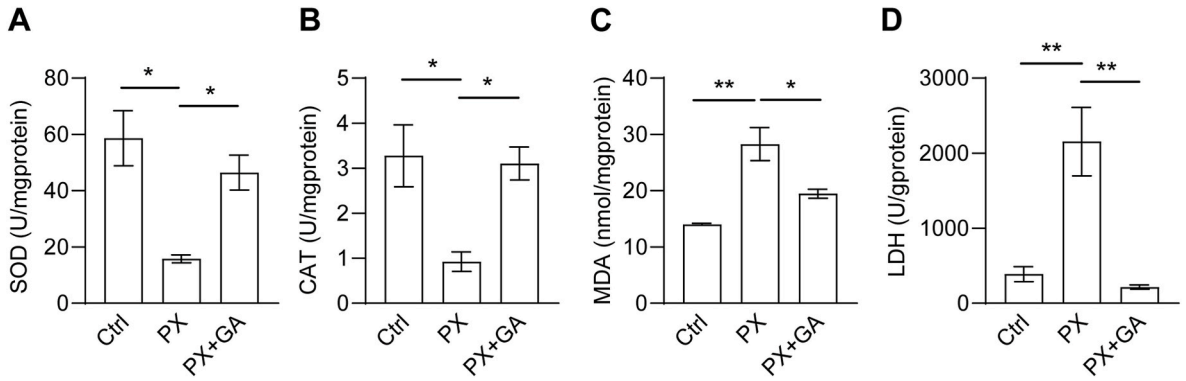

Fig. 3. The impact of PX exposure on enzyme activity. (A–B) Summarized data showing decreased activities of SOD (A) and CAT (B) following PX exposure; co-treatment with GA reversed these effects (A, B). (C–D) PX-induced increase in MDA (C) and LDH (D) activities were attenuated by GA co-treatment. N = 3, 3, 3 for Ctrl, PX and PX + GA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

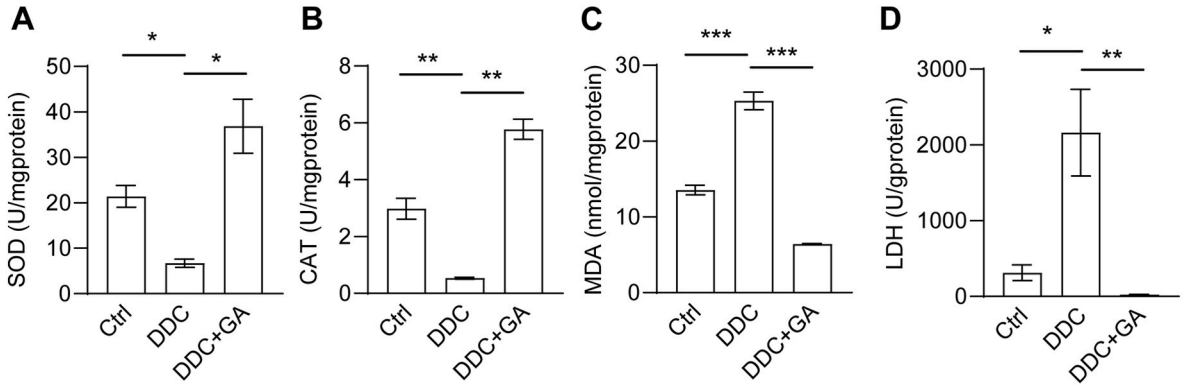

Fig. 4. DDC exposure decreases enzyme activity. (A–D) Summary of data showing that the activities for SOD (A, N = 4,4,4), CAT (B, N = 3,3,3), MDA (C, N = 3,3,3) and LDH (D, N = 3,3,3) in control, DDC-treated and DDC + GA-treated optic tectum. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

CAT activity (Fig. 4B), further suggesting that PX may increase cell toxicity primarily through oxidative stress pathway. Furthermore, increased activities of MDA and LDH induced by DDC were effectively mitigated by GA co-treatment (Fig. 4C–D). These findings imply that DDC exposure caused oxidative stress and cell damage by reducing antioxidant enzyme activities and increasing markers of cell injury and GA may have protective agent against oxidative stress.

3.4. GA mitigates DDC-induced neural apoptosis

To study the toxic effect of DDC on the optic tectum, the tadpoles were incubated with DDC (50 nM) for 72 h and used to measure the number of apoptotic cells (Fig. 5A). All TUNEL-positive apoptotic cells were counted from the whole tectum. Our data demonstrated a marked increase in apoptotic cells in the optic tectum of DDC-treated groups (Fig. 5B). However, co-treatment with GA for 72 h significantly reduced the number of apoptotic cells comparable to controls (Fig. 5B). These results highlight GA's capacity to mitigate cell apoptosis by counteracting oxidative damage induced by DDC.

3.5. GA co-treatment facilitates behavioral recovery

To determine whether PX exposure impairs tadpole behavior, we incubated animals in PX for 72 h and measured the swimming speed and distance. The swimming movements of the tadpoles over a 1 min were recorded and analyzed using the behavioral setup (Fig. 6A). Our results

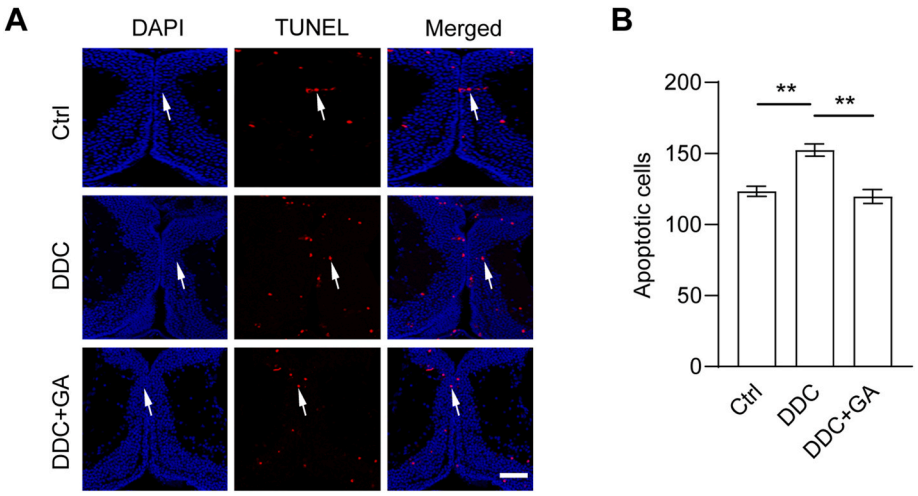

Fig. 5. Numbers of apoptotic cells are increased with DDC exposure. (A) Representative images of apoptotic cells in the optic tectum of control (top), DDC-exposed (DDC, 2 mM, middle) and DDC + GA (GA, 5.7 mM) co-treated (bottom). Dotted lines outline the optic tectum and arrows indicate apoptotic cells. Scale bar: 30 μm. (B) Summary of data showing that the number of apoptotic cells was significantly increased in DDC-treated animals, which was prevented by GA co-treatment. N = 4, 5, 5 for Ctrl, DDC and DDC + GA. $^{**}P < 0.01$.

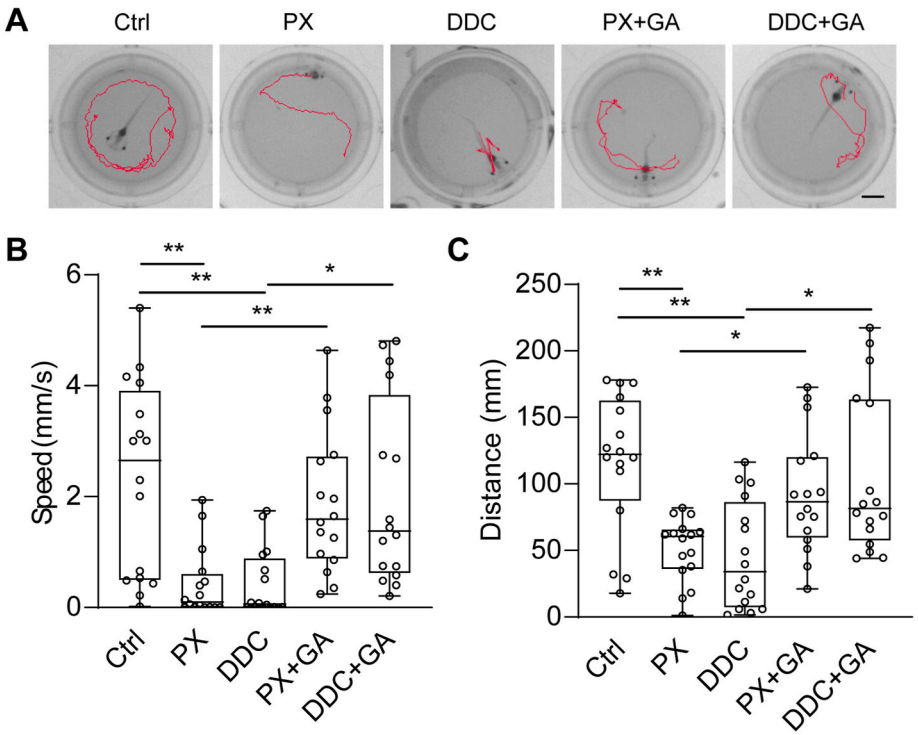

Fig. 6. GA co-treatment rescues the behavioral impairment induced by PX or DDC exposure. (A) A schematic representation of tadpole swimming behavior across six experimental groups: Ctrl, PX, PX + GA, DDC, DDC + GA and GA. Scale bar: 2 mm. (B) Summary of data showing that the swimming speed (mm/s) and distance (mm) were significantly decreased in DDC-exposed tadpoles, which were reversed by GA co-treatment. N = 16, 16, 16 for Ctrl, PX, DDC, PX + GA and DDC + GA. $^{*}P < 0.05$, $^{**}P < 0.01$.

showed that both swimming speed and distance were dramatically decreased after PX treatment, suggesting impaired motor function. Importantly, GA co-treatment restored these behavioral measures to levels observed in the control group (Fig. 6B and C). Similarly, GA was able to reverse the swimming deficits induced by DDC treatment (Fig. 6B and C). These results further support GA's neuroprotective role by demonstrating its ability to counteract oxidative damage and restore normal motor function.

4. Discussion

This study investigated the neurotoxic effects of *para*-xylene (PX) exposure in *X. laevis* by analyzing transcriptomic changes and oxidative stress markers in the optic tectum. Our findings indicate that PX exposure induced significant oxidative stress in the optic tectum, leading to widespread transcriptional alterations, including downregulation of key antioxidant genes (*sod1.L*, *gsr.L*, *cat.L*) and upregulation of *GPx3.L*. Enzymatic assays confirmed reduced SOD and CAT activity, along with increased TBARS and LDH levels, indicating oxidative damage and

cellular toxicity. Inhibition of SOD with DDC replicated these effects, further implicating SOD signaling in PX-induced neurotoxicity. GA co-treatment effectively restored antioxidant enzyme activity, reduced apoptosis, and mitigated behavioral impairments, highlighting its neuroprotective potential against PX-induced oxidative damage.

While PX is a well-documented neurotoxin affecting the central nervous system (Gao et al., 2016; Liao et al., 2020), its impact on transcriptional regulation are not fully understood. The tectum is essential for visual processing and related behaviors (Dong et al., 2009; Shen et al., 2011). In our study, tadpoles were exposed to neurotoxins during a critical developmental stage when neural circuits are forming, making this period particularly vulnerable to abnormal gene expression (James et al., 2015). Previous studies have shown that PX modulates cell survival and alters transcriptional regulation by changing histone acetylation in the optic tectum (Gao et al., 2016; Liao et al., 2020), suggesting that these changes in gene expression may contribute to its neurotoxic effects. Using transcriptome sequencing, we conducted a comprehensive gene expression analysis of gene expression at the RNA level in PX-exposed larvae. We analyzed the pathways through which PX-induced neurotoxicity and provided a theoretical basis for its underlining mechanism. GO enrichment analysis highlighted significant disruptions in cellular processes, including cell communication, signal transduction, regulation of biological process and cellular response to stimulus. Additionally, KEGG pathway analysis implicated efferocytosis, MAPK signaling and FoxO signaling pathways, reinforcing the role of oxidative stress in PX-induced neurotoxicity. These results align with prior research on other benzene-derived compounds (Costa et al., 2006; Neuparth et al., 2014), further validating the use of *X. laevis* as a model organism for studying PX neurotoxicity and identifying potential gene targets. The integration of high-throughput RNA-seq not only aids in the discovery of novel biomarkers and therapeutic targets but also helps clarify the molecular pathways that contribute to the observed behavioral deficits.

Subsequent analyses confirmed the expression of oxidative stress-related genes using qRT-PCR, which supported the RNA-seq findings. Notably, genes such as *sod1.L*, *gsr.L* and *cat.L* showed increased expression following PX exposure, and the corresponding enzyme activities of SOD and CAT mirrored these changes. This suggests that even very young *X. laevis* tadpoles possess functional antioxidant systems. Furthermore, since GPx enzymes are responsible for converting hydrogen peroxide and lipid peroxides into non-toxic molecules (Buryskova et al., 2006; Matés, 2000), the elevated expression of *GPx3.L* likely represents a compensatory cellular response to oxidative stress. In scenarios where SOD or CAT pathways are compromised, GPx3 can serve as an alternative route to mitigate peroxidative injury (Lubos et al., 2011). The selective upregulation of *GPx3.L* may therefore indicate a shift in antioxidant strategy, favoring glutathione-dependent detoxification under PX-induced stress conditions. Similar compensatory induction of GPx genes has been reported in models of oxidative damage and environmental pollutant exposure (Amstad et al., 1994; Nunes et al., 2017), and there is evidence to suggest that *GPx3* may be regulated as a Yap1-target gene (Yologlu and Ozmen, 2015). It is also important to note that antioxidant defense responses vary across species and depend on toxicant concentration (Matés, 2000). These findings suggest that while PX suppresses several primary antioxidant defenses, the enhancement of *GPx3.L* expression may represent an adaptive cellular attempt to restore redox balance in the developing brain. Overall, the coordinated alterations in gene expression and enzyme activity underscore the potential of these biomarkers in assessing BTEX-related toxicity, emphasizing the importance of evaluating multiple antioxidant responses to better understand toxicant-induced stress.

In biological systems, SOD catalyzes the conversion of superoxide anions into hydrogen peroxide, which is subsequently detoxified by enzymes such as CAT and GPx (Dumay et al., 2006). Lipid peroxidation (LPO) is a hallmark of oxidative stress-induced cellular damage, with MDA, a byproduct of LPO, serving as a widely recognized biomarker of oxidative injury (Buryskova et al., 2006; Chen et al., 2024b). Additionally, LDH release reflects cell membrane integrity and cytotoxicity. Our enzymatic assays demonstrated that PX exposure led to a reduction in SOD and CAT activity, along with elevated TBARS and LDH levels, reinforcing the conclusion that oxidative stress contributes to tectal cell injury in PX-induced neurotoxicity. Furthermore, inhibition of Cu/Zn-SOD with DDC led to increased superoxide accumulation and reduced downstream detoxification capacity handled by CAT, thereby compromising the cellular antioxidant defense system and exacerbating oxidative stress-induced cell apoptosis (Rahden-Staroń et al., 2012). Importantly, concomitant treatment with GA successfully restored enzymatic activity and reduced apoptotic cells (Gao et al., 2016), suggesting its protective role may be mediated through modulation of oxidative stress pathways by enhancing SOD function and glutathione levels (Yu et al., 2018). Given that previous studies have demonstrated the ability of antioxidants to prevent ROS-induced cell death, GA may serve as a potential therapeutic agent for mitigating oxidative damage associated with neurotoxicant exposure (Dumay et al., 2006; Saeidnia and Abdollahi, 2013).

Behavioral assessment is a critical element in evaluating neurotoxicity, as it provides insights into the functional impact of toxicants on the nervous system (Chen et al., 2024b; Gao et al., 2016; Gao and Shen, 2021; Ge et al., 2021; Liao et al., 2020; Spawn and Aizenman, 2012). Oxidative stress not only causes cellular damage but also leads to behavioral impairments (Bushnell, 1988; Nunes et al., 2017; Spawn and Aizenman, 2012). In this study, behavioral assays revealed that both PX and DDC significantly suppressed locomotor activity in tadpoles, as evidenced by reduced swimming speed and distance. These effects are likely attributable to impaired synaptic transmission and abnormal tectal establishment, leading to deficits in sensory integration and disrupted animal behavior (Gao et al., 2022; James et al., 2015; Liao et al., 2020). Furthermore, DDC exposure was associated with marked apoptosis in the optic tectum, suggesting that excessive ROS generation may compromise neural network integrity and contribute to the observed deficits in larval swimming behavior (Spawn and Aizenman, 2012). In contrast, co-treatment with GA notably restored locomotor activity, indicating that GA may mitigate neurotoxic effects through its antioxidant properties, potentially by rescuing synaptic transmission and protecting the CNS from oxidative damage (Liao et al., 2020). However, this study focused exclusively on the optic tectum and future studies involving additional areas of the *Xenopus* system are needed to determine the broader applicability of these results.

In summary, our study demonstrates that PX exposure induces significant oxidative stress and neurotoxicity in the optic tectum of *X. laevis* larvae, as evidenced by reduced activities of SOD and GR, along with increased activities of MDA and LDH. The neurotoxic effects replicated by DDC confirm the critical role of SOD in antioxidant defense, while the protective effects of GA highlight its potential as a therapeutic agent. These findings advance our understanding of PX-induced neurotoxicity and suggest that targeting antioxidant pathways, particularly those involving SOD, could offer promising strategies for intervention.

CRediT authorship contribution statement

Lu Han: Supervision, Project administration, Methodology, Investigation. **Wenyi Huang:** Methodology, Formal analysis, Data curation. **Lingyun Meng:** Software, Methodology, Data curation. **Xinyi Duan:** Software, Methodology, Data curation. **Yufei Wu:** Methodology, Data curation. **Qihui Lin:** Methodology, Data curation. **Xiaohua Wu:** Supervision, Methodology, Data curation. **Qi Chen:** Supervision. **Wanhua Shen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they 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.envpol.2025.126756.

Data availability

Data will be made available on request.

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