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Prolonged exposure to the synthetic glucocorticoid dexamethasone induces brain damage via oxidative stress and apoptotic response in adult *Danio rerio*

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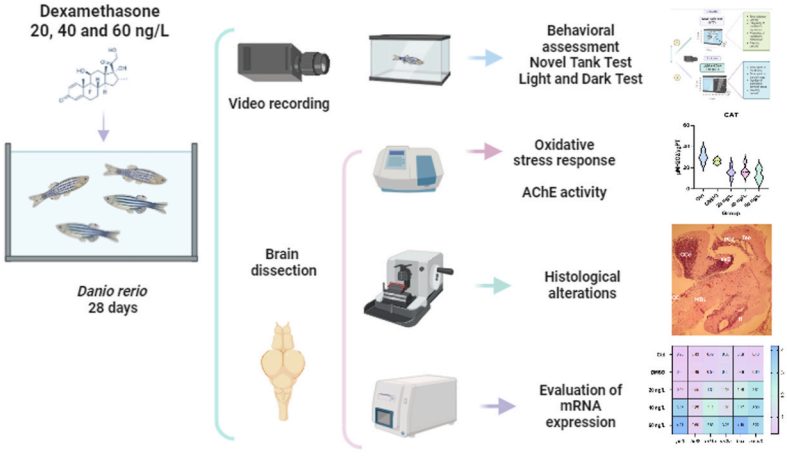
HIGHLIGHTS

- Subchronic exposure of *Danio rerio* to DEXA induces biochemical and behavioral toxicity.
- DEXA is known to cause anxiety-like symptoms and alter exploratory behavior in zebrafish model.
- Inhibition of AChE activity in the brains of adult zebrafish suggests neurotoxicity.
- DEXA exposure led to brain damage, including perineuronal vacuolization and cell engulfment.
- The upregulated genes were linked to both phenotypic and behavioral responses.

GRAPHICAL ABSTRACT



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ABSTRACT

Due to its extensive use as a painkiller, anti-inflammatory, and immune modulatory agent, as well as its effectiveness in treating severe COVID-19, dexamethasone, a synthetic glucocorticoid, has gained attention not only for its impact on public health but also for its environmental implications. Various studies have reported its presence in aquatic environments, including urban waters, surface samples, sediments, drinking water, and wastewater effluents. However, limited information is available regarding its toxic effects on nontarget aquatic

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Oxidative stress
Apoptotic response

organisms. Therefore, this study aimed to investigate the mechanism of toxicity underlying dexamethasone-induced brain damage in the bioindicator *Danio rerio* following long-term exposure. Adult zebrafish were treated with environmentally relevant concentrations of dexamethasone (20, 40, and 60 ng L-1) for 28 days. To elucidate the possible mechanisms involved in the toxicity of the pharmaceutical compound, we conducted a behavioral test battery (Novel Tank and Light and Dark tests), oxidative stress biomarkers, acetylcholinesterase enzyme activity quantification, histopathological analysis, and gene expression analysis using qRT-PCR (*p53, bcl-2, bax, caspase-3, nrf1,* and *nrf2*).The results revealed that the pharmaceutical compound could produce anxiety-like symptoms, increase the oxidative-induced stress response, decrease the activity of acetylcholinesterase enzyme, and cause histopathological alterations, including perineuronal vacuolization, granular and molecular layers deterioration, cell swallowing and intracellular spaces. The expression of genes involved in the apoptotic process (*p53*, *bax*, and *casp-3*) and antioxidant defense (*nrf1* and *nrf2*) was upregulated in response to oxidative damage, while the expression of the anti-apoptotic gene *bcl-2* was down-regulated indicating that the environmental presence of dexamethasone may pose a threat to wildlife and human health.

1. Introduction

The coronavirus disease (SARS-CoV-19), a pneumonia-like disease, emerged in December 2019 in China and quickly spread worldwide, with implications not only on public health and economic activities, but also on the environment (Kuroda et al., 2021; Mehta et al., 2021). Treatment guidelines for the therapeutic management of severe inflammation caused by COVID-19 involve the use of mechanical ventilation and the administration of synthetic glucocorticoids (GCs) (Aranda et al., 2023). Glucocorticoids (GCs) are a well-known group of endocrine-disrupting steroids that regulate several physiological and metabolic functions under both normal and stress conditions (Vincken et al., 2022). Dexamethasone (DEXA), a synthetic glucocorticoid, is considered one of the most effective therapeutic treatments for COVID-19 patients because of its anti-inflammatory activity, low cost, and wide availability (Ahmed and Hassan, 2020; Rodriguez-Martinez et al., 2020). DEXA has also been widely used to treat various conditions, including myeloma, meningitis, postoperative vomiting and nausea, pulmonary and brain edema, psoriasis, and both acute and chronic pain (Fotiou et al., 2022; Jiang et al. 2021; Liu et al., 2020; S. Liu et al., 2022; Wang et al., 2022). Its mode of action is mediated via the GC receptor through the transactivation of diverse anti-inflammatory cytokines and transrepression of pro-inflammatory cytokines (Ingawale and Mandlik, 2020). Dexamethasone has relatively low absorption rates, typically ranging from 50 to 90% (Herrero et al., 2012; Sulaiman et al., 2014). Its main metabolite is 6β-hydroxydexamethasone as well as 6α-hydroxydexamethasone, and their biotic and abiotic transformations are not well understood (Gu et al., 2021; Pervaiz et al., 2015). According to a report by Polaris Market Research, the market for corticosteroids, including GCs, is predicted to experience a growth rate of >4.6% between 2024 and 2032, leading to a significant increase in environmental occurrence (Polaris Market Research, 2023). For instance, concentrations ranging from ng/L to μg/L have been detected in diverse aquatic matrices, including effluents from industrial, hospital, and wastewater treatment plant (WWTP) facilities, surface and drinking water, sediments, and soils (Creusot et al., 2014; Praveena et al., 2018; Shen et al., 2020). This GC has emerged as an emerging contaminant in aquatic ecosystems because of its inability to be eliminated during wastewater treatment and its lipophilic, persistent, and bioaccumulative properties that can cause noxious effects on both aquatic and terrestrial ecosystem (Barquín et al., 2024). Studies have suggested that organisms can be affected by the presence of DEXA in their environment leading to embryotoxicity and teratogenic defects (Di Paola et al., 2022), histopathological alterations (Cuzziol Boccioni et al., 2020), immunological and hematological damage (Sayed et al., 2022), changes in gene expression (Zhong et al., 2021a), and exacerbation of oxidative stress responses (Guiloski et al., 2015a). DEXA resembles cortisol, a hormone that binds to glucocorticoid receptors (GR) to control genes essential for development, immune response, osmoregulation, glucose metabolism and behavior (Nesan and Vijayan, 2013; Skupio et al., 2015; Takahashi and Sakamoto, 2013; Viana et al., 2006). In this sense, due consideration ought to be accorded to behaviors that arise in response to stressful circumstances, as they play a vital role in facilitating adaptive responses to environmental changes. Employing behavioral bioassays that quantitatively and qualitatively assess the behavior of organisms to evaluate and analyze internal indicators of physiological or psychological stress in response to external stimuli is essential to understand the possible mechanisms involved in brain damage in diverse bioindicators, including zebrafish (Orozco et al., 2022). Zebrafish are widely regarded as a leading model for investigating the processes underlying the effects of neurotoxic compounds, including biochemical, molecular, and genetic evaluation (D'Amora and Giordani, 2018). A growing body of evidence has highlighted the diverse potential hazards associated with DEXA in humans and other rodent models. However, its neurotoxic effects at environmentally relevant concentrations in aquatic organisms have not been investigated. To the best of our knowledge, no scientific research has been conducted on molecular, cellular, tissular and organismal approaches to comprehend the neurobehavioral effects of DEXA in adult zebrafish, although, there is a substantial body of knowledge about cell loss, diminished neurogenesis, glial proliferation, and, altered behavior as well as the suppression of brain growth and the resulting structural and functional abnormalities in other biological models (Ahmed, 2016; Chen et al., 2010; de Kloet et al., 2014).

This study aimed to determine the impact of three different environmentally relevant concentrations of dexamethasone (20–60 ng/L) on the behavior of zebrafish adults after 28 days of exposure, utilizing the novel tank and dark and light-dark tests. Additionally, we assessed the expression of genes associated with the response to free radicals and apoptotic processes (nrf1, nrf2, bax, bcl2, casp3, and p53) as well as biomarkers for oxidative stress, antioxidant response, and acetylcholinesterase activity. Histological analyses were performed to observe cellular changes in brain structure. Our findings reveal an intriguing phenomenon in which subchronic exposure to a highly consumed glucocorticoid at concentrations ranging from ng/L may selectively evoke biochemical, behavioral, or physiological responses. Moreover, our data underscores the reliability of the NTT and LDT as sensitive tools for assessing anxiety-related behaviors in adult zebrafish.

2. Methods

2.1. Experimental concentrations of dexamethasone

Dexamethasone (CAS number 50-02-2 analytical standard, purity >98%) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Louis, MO, USA). Similar to all the reagents used for oxidative stress, AChE activity, gene expression, and histological evaluation. The experimental concentrations used in this study were 0, 20,40,60 ng/L (nominal concentrations). Concentrations were established based on Gutiérrez-Noya et al. (2023) and Onofre-Camarena et al. (2024) and are representative of environmental levels. Solutions were prepared in DMSO 0.01%. Also, a DMSO (0.01%) control system was employed.

2.2. Fish procurement

Adult wild-type zebrafish were housed in 60-L tanks for 45 days with dechlorinated tap water, under the following conditions: temperature at 27 ± 1 °C, pH ranging from 7.1 to 7.7, oxygen saturation exceeding 70%, and a 12-h dark/light photoperiod. The fish were fed twice daily with dry flakes according to the method established by (Ferdin and Halili, 2017) and the recommendations outlined in the OECD Guidelines (Guidelines et al., 2013). All physical and chemical conditions of the water remained constant and were monitored during both acclimation and exposure periods.

2.3. Experimental protocol

Thirty individuals per concentration were placed in five 10-liter tanks for exposure to various concentrations of DEXA (0, 20, 40, and 60 ng/L) and DMSO system based on the recommendations of (Ferdin and Halili, 2017). The fish were fed twice daily, and water was renewed every 72 h with normal cleaning. After exposure, the specimens were used to perform non-lethal analyses (behavioral tests) and then anesthetized on ice, and their brain tissues were removed. Each sample consisted of tissues from three fish.

2.4. Behavioral test battery

2.4.1. Novel Tank Test (NTT)

After 28 days of exposure, behavioral trials were conducted using the methodology described by Cachat et al. (2010). This entailed transferring fish from the exposure tanks to an isolated room containing the behavioral chamber, allowing them to become accustomed to their new surroundings for an hour. Subsequently, one fish was randomly selected and placed in a 5 L tank for an 8-min period, with the first 2 min used for acclimation and the remaining 6 min dedicated to the trial. Ethovision XT 13.0 software was used to record fish behavior and measure various anxiety endpoints: freezing time (s), total distance traveled (cm), mean speed (cm/s), total distance traveled at the top and bottom (cm), latency at the top (s), total number of transitions from top to bottom, and vice versa, and total time spent in the top and bottom sections. All behavioral tests were performed between 10:00 and 15:00 h. We ensured the presence of 1:1 males and females per concentration for each trial.

2.4.2. Light/dark test (LDT)

Briefly, after NTT, the light-dark transition (LDT) was conducted in a tank that was divided into two equal sections, with one side featuring bright white walls and a floor and the other side having a black partition; there were no physical barriers separating the two compartments, nor was there a starting box or lid positioned over them. Six animals per concentration (triplicates, $n = 18$) were individually placed in the behavioral device, and their activity was recorded and analyzed for a period of 8 min (2 min for acclimation and 6 min for the trial). The Tox Track Ink software system was used to measure the time spent in the light and dark areas, the transitions between the two zones, and the freezing periods. This analysis was performed according to the methodologies described by Adedara et al. (2022) and Fontana et al. (2022) with slight modifications.

The experimental design showing the behavioral test battery can be seen in Fig. 1.

After conducting the Light and Dark Test, we followed the American Veterinary Medial Association Guidelines on Euthanasia (AVMA, 2020) by placing each fish individually in a 50 mL beaker to induce hypothermic shock. We determined the fish's death by closely observing the complete cessation of opercular gill movement and the absence of any response to tactile stimulation. The fish were then dissected, and the brains were extracted for each analysis (oxidative stress, AchE activity, histopathological alterations, and gene expression). The behavioral trials were conducted in triplicate to calculate the mean.

2.5. Quantitation of oxidative stress biomarkers

A total of 18 brains per concentration were weighed, transferred to 1.0 mL vials filled with phosphate buffer (pH 7.4), and homogenized. The organs were suspended in tubes containing buffer solution (pH 7.4), homogenized, centrifuged at 12,500 rpm at a temperature of −4 ± 0.5 °C for 15 min, and then stored at −80 ± 2 °C. Half of the brains were used in oxidative stress analyses, and half were reserved for the determination of AChE activity. The supernatant was used to determine various biomarkers, including lipoperoxidation (LPX), using the TBARS methodology (Buege and Aust, 1978) and hydroperoxide content (HPX), according to the methodology of Jiang et al. (1992), superoxide dismutase activity (Marklund and Marklund, 1974), catalase activity based on the method described by Radi et al. (1991), and total protein content

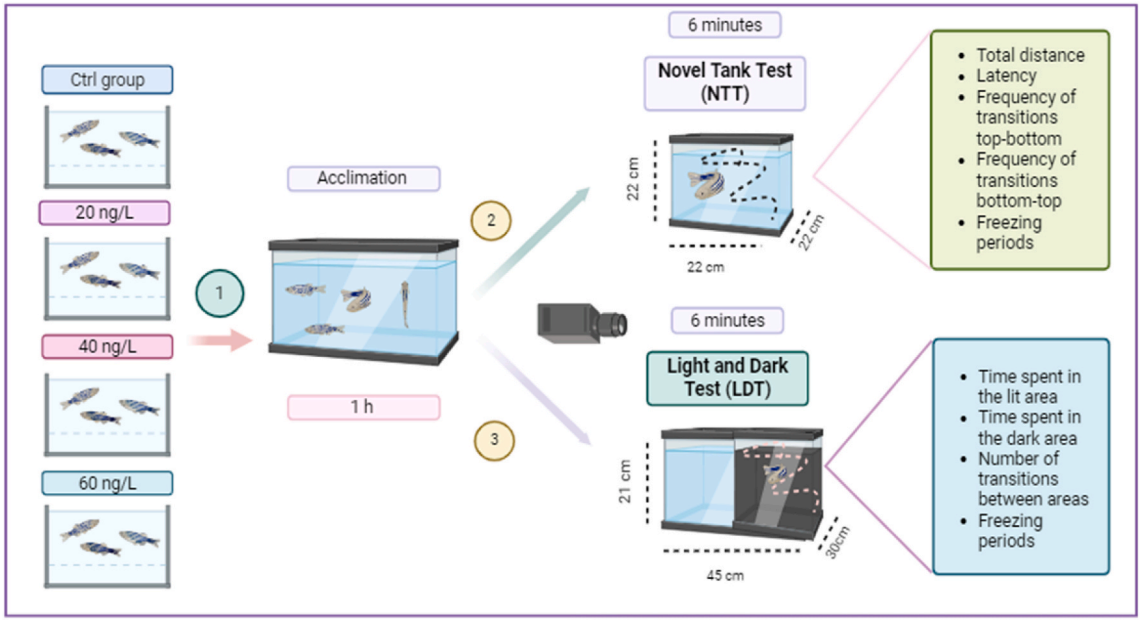

Fig. 1. Schematic representation of the experimental design showing the behavioral battery tests.

(TP) to normalize the biomarkers. The protein carbonyl content (PCC) Stadtman and Levine (2003) was determined using the precipitate.

2.6. AChE activity evaluation

Three brain samples (triplicate) that were stored at each concentration were used to assess acetylcholinesterase (AChE) activity, according to a protocol developed by Ellman et al. (1961). To accomplish this, we combined 400 μL of the supernatant with 2.6 mL of phosphate buffer (pH 8.0, 0.1 M) and 0.1 mL of DTNB (5,5-dithiobis-2-nitrobenzoate, 0.1 M). The changes in absorbance were measured every minute for 5 min at 412 nm.

2.7. Evaluation of mRNA expression

Three brain samples (triplicate) from each concentration and the control group were thawed in an ice bath. RNA was extracted using the RNeasy Mini Kit (QIAGEN, Hilden, Germany). To confirm the quality of the RNA, agarose gel electrophoresis (1%) was performed and a NanoDrop 2000/2000c (Thermo Scientific, USA) spectrometer was used to ensure purity. All samples were stored at −22 °C. Isolated RNA samples were thawed in an ice bath for reverse transcription. In sterile eppendorf tubes (RNases free), the following reactants were added: 2.0 μL of 7x gDNA Wipeout Buffer, 2.0 μL of RNase-free water (both from the QIAGEN QuantiTect Reverse Transcription Kit), and 10.0 μL of RNA. The mixture was incubated for 2 min at 42 °C and then cooled.

The cooled samples were combined with 1.0 μL of Quantiscript reverse transcriptase, 4.0 μL of Quantiscript RT Buffer 5x, and 1.0 μL of RT Primer Mix (QIAGEN QuantiTect Reverse Transcription Kit). The mixture was incubated at 42 °C for 15 s followed by incubation at 93 °C for 3 min. Following reverse transcription, the quality of the complementary DNA (cDNA) was determined by electrophoresis and spectrophotometry, as previously described.

The qPCR reaction was performed by adding 25 μL of 2 × QuantiTect SYBR Green PCR (QIAGEN), 1.0 μL of primer, 4.0 μL of cDNA, and 19 μL of RNase-free water. All reagents were mixed, and qPCR was performed under the following conditions: 10 min at 94 °C, at the same temperature 35 cycles of denaturation for 15 s, primer annealing for 30 s, and extension for 30 s at 72 °C. QIAGEN Gene Q Rotor qPCR was used. The relative quantification of the target gene was normalized to that of β-actin using the 2−ΔΔ Ct method (Pfaffl, 2001; Schmittgen and Livak, 2008). The table below (Table 1) displays the primer sequences used for evaluating gene expression in the zebrafish brain.

2.8. Histological alterations

After the 28-day exposure to DEXA, three fish (triplicate) were anesthetized using the ice-cold method and their heads were removed. The heads were fixed in Davidson's solution for 15 d. Afterwards, a 15-day period of decalcification (HCl 5%) was performed. All solutions were volume 10X with daily medium renewal. The decalcified heads were dehydrated, clarified, infiltrated in alcohols of different grades and xylol, and then embedded in kerosene. The samples were embedded in paraffin, and the fish were oriented sagittally. Paraffin-embedded samples were sectioned (5 mm thick) with a CUT 5062 microtome (SLEE ®) and stained with hematoxylin and eosin (H&E). The tissue preparations were analyzed under a MOTIC® optical microscope (model BA410), where photomicrographs of the area of interest were taken. To characterize the structure of the brain and the presence or absence of histological changes, tissue samples were observed at magnifications of 10X, 40X and 100X for each histological sample obtained. Histopathological analysis included preparation of samples, observation and description of pathological alterations according to the method provided by Onofre-Camarena et al. (2024).

Table 1
Sequences of the evaluated genes.

Gene	Primer sequence	Reference
p53	**F**: CTTGGTGCTGAATGGACAAC **R**: CCAGAGTGATGATTGTGAGGA	Umamaheswari et al. (2021)
bcl2	**F**: AGGAAAATGGAGGTTGGGAT **R**:GTTAGGTATGAAAACGGGTGG	Qian et al. (2018)
nrf1a	**F**: TTTGGTTCCCGATGAAGAC **R**:TGATTAGCGTGAGACTGAGC	Sant et al. (2017)
nrf2a	**F**: GAGCGGGAGAAATCACACAGAATG **R**: CAGGAGCTGCATGCACTCATGC	Williams et al. (2013)
bax	**F**: GGCTATTTCAACCAGGGTTCC **R**: TGCGAATCACCAATGCTGT	Wang et al. (2020)
caspase 3	**F**: GACGTGAAACCAAAGGGTGT **R**: TGATTTGGCATCCATCTGAA	Gaaied et al. (2022)
β- *actin*	**F**:GCCAACAGAGAGAAGATGAC **R**: CACCAGAGTCCATCACAATAC	Umamaheswari et al. (2021)

2.9. Concentrations of DEXA in zebrafish brain

To evaluate DEXA concentrations in exposed water and brains of *D. rerio*, a two-step filtration process using 8 μm and 2.5 μm membranes, followed by Solid-Phase Extraction (SPE) cartridges from Phenomenex Strata XL in Torrance, CA, was performed. SPE cartridges were prepared by conditioning the cartridges with 10 mL methanol (MeOH) and 10 mL milli Q® water. Initially, water samples that had been spiked with DEXA were introduced into cartridges (5 mL/min). Thereafter, the samples were eluted using methanol (6 mL). Subsequently, the solvent was evaporated to dryness using N-Evap nitrogen from Organomation (Haverhill, MA, USA). Afterwards, the dried samples were reconstituted with 1 mL of a solution of MeOH and water (50:50) and passed through a filter of 0.2 μm before being injected. During the examination of the zebrafish brain to determine DEXA levels, the tissue was subjected to a water wash and drying process. A 0.3 g portion of the brain tissue was mixed with a DEXA solution, and then a 30-s vortexing process was performed using a Jeho Tech VM-96B vortexer (Korea). The DEXA-enriched sample was stored at −20 °C for 30 min to initiate the extraction process. Twenty milliliters of a MeOH and milli Q® water mixture (1:1) was added to the sample, which underwent a 10-min extraction using a Nexul NXP 1002 ultrasonic cleaner (Japan). Finally, the sample was centrifuged at 24000 rpm for 10 min to ensure the thorough separation of the organic phase. Afterwards the SPE (Solid Phase Extraction) cartridge was conditioned with a combination of 5 mL of MeOH, 5 mL of ultrapure water, and 5 mL of a 1:1 mixture of MeOH and ultrapure water. After adding the sample, the cartridge was rinsed with 5 mL of ultrapure water and dried under vacuum for 15 min. To elute the sample, 10 mL methanol was initially used, followed by 5 mL of a 1:1 mixture of acetone and methanol. The cartridge was then dried under vacuum for 3 min. The eluent was then concentrated using an evaporator and dried under a nitrogen flow. Subsequently, the concentrated eluent was reconstituted with three parts acetonitrile and seven parts ultrapure water (1 mL) and filtered through a 0.2-μm PTFE membrane filter. The measurement of DEXA levels in the exposure water and the assessment of the brains were carried out using a Liquid Chromatography-Mass Spectrometry (LC-MS/MS) system from Waters (Milford, Massachusetts). The analysis was conducted using an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in MeOH (B). The column temperature was maintained at 40 °C, with an injection volume of 10 μL and a flow rate of 0.3 mL per minute. Moreover, the run time was set at 10 min, starting with 90% A at 0.1 min and transitioning to 10% A at 8 min, and ultimately returning to 90% A at 10 min. Exposure water and brain samples were analyzed using a Waters Premier XE triple quadrupole mass spectrometer (Milford, Massachusetts USA) with electrospray ionization in positive mode. The mass spectrometry settings used for quantification were as follows: positive ion mode, parent ion (m/z) = 363.3, product ion (m/z) = 147.3, and cone voltage (V) = 22. The detection limit (LOD) for water was 1.6 ng/L,

with a quantification limit (LOQ) of 3.42 ng/L. For brain samples, the LOD was 0.09 ng/g, and the LOQ was 0.52 ng/g. To evaluate the matrix effect, the gradients derived from the calibration curves fitted to the matrix were compared with those obtained from the solvent standards. The bioconcentration factor (BCF) was calculated using the concentration of DXE in both the brain and exposure water, as per the formula (Garcia-Medina et al., 2022):

BCF = (brain concentration) / (exposure water concentration)

2.10. Statistics

Kolmogorov-Smirnov and Bartlett's tests were used to evaluate the normality and homogeneity of variances, respectively. Two-way ANOVA with multiple testing was performed to analyze the results, which were expressed as the mean ± standard error of the mean (S.E.M). The Tukey test was used for post-hoc analysis, comparing all groups. Statistical significance was set at $P \leq 0.05$. Pearson's correlation analysis was used to examine the association between the behavioral tests and biomarkers.

Ethical approval

The current research was meticulously scrutinized by the Ethics and Research Committee of the Autonomous University of the State of Mexico (approval ID: RP.UAEM.ERC.040.2023) to ensure optimal experimental conditions and uphold superior animal care standards. Additionally, the guidelines outlined in NOM-062-ZOO-1999 were strictly adhered to for the purposes of breeding, care, and experimental use of animals.

3. Results

3.1. Behavioral tests

3.1.1. NTT

The Novel Tank Test phenotypes are presented in Fig. 2. According to our results, DEXA induced hypolocomotion with a statistically shorter total distance moved, which signified a reduction in the exploratory behavior of the treated fish during the 6 min period of the test. Furthermore, it was observed that as the concentration of DEXA rose, the thigmotaxis displayed a similar pattern, with the duration at the top of the tank decreasing and the latency time at the top significantly increasing indicating alterations in the natural seeking behavior of fish. This resulted in a reduction in the frequency at both the top and bottom, as well as a decrease in the bottom-top transitions. DEXA significantly affected the mobility of fish, decreasing the high mobility duration and increasing the immobility duration, indicated by prolonged freezing periods compared with that in the non-exposed individuals. These results suggest that fish experience an anxiety-like state and stress owing to prolonged exposure to environmentally relevant concentrations of DEXA.

3.1.2. LDT

The Light/Dark Test is a valuable resource for assessing the behavioral response of free-swimming organisms in terms of their activity levels in lit or dark areas as well as the transitions that occur between these two environments (Irons et al., 2010). This test was designed to provide insights into how organisms react to different light conditions after prolonged exposure to synthetic glucocorticoids. Our results showed that the control and DMSO groups spent more time on the dark side, whereas the treated groups (mainly 40 and 60 ng/L) spent most of the time on the lit side (this difference was statistically significant,

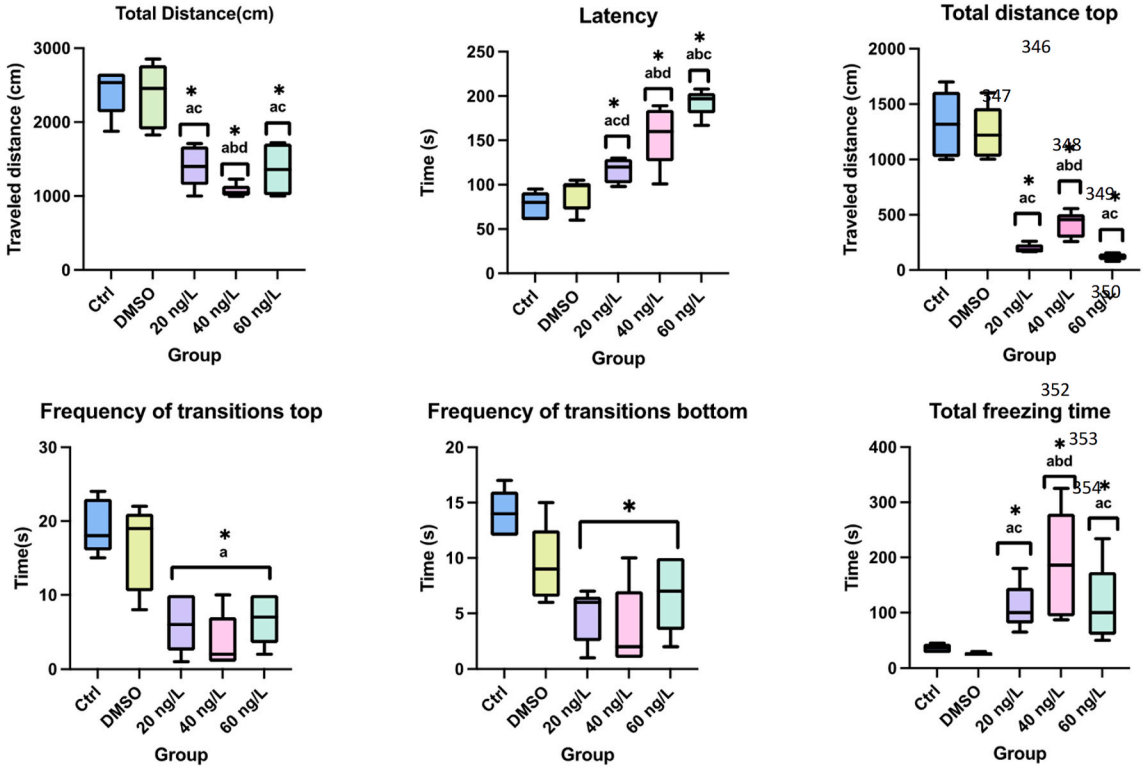

Fig. 2. *D. rerio* adults' behavioral patterns observed in the Novel Tank Test after 28 d of exposure to DEXA. The values represent the mean of three replicates ± SEM. *Significative differences regarding the non-exposed (Ctrl and DMSO) groups. Letters show statistically different values compared to specimens exposed to [a] DMSO, [b]20 ng/L, [c]40 ng/L, [d]60 ng/L. Two-way ANOVA $p<0.05$. Total distance moved ($F_{(2.82)=}24.35$; $p<0.05$); Latency time ($F_{(2.82)=}65.80$; $p<0.05$); Frequency at the top and bottom ($F_{(2.82)=}18.62$; $p<0.05$); Bottom-top transitions ($F_{(2.82)=}18.31$; $p<0.05$); Freezing period ($F_{(2.82)=}16.65$; $p<0.05$).

showing dark-avoidance behavior). This behavior is known as scotophobia (Brodrick and Jékely, 2023). In addition, the frequency of transitions between sides was significantly lower in the treated samples than in the non-exposed groups, indicating a neophobic response to rapid changes in the environment. The freezing periods in the light/dark areas were significantly higher than those in the control groups. Statistical differences were also observed between groups, mainly between 20 and 60 ng/L (Fig. 3).

3.2. Oxidative stress biomarkers

This study aimed to analyze biomarkers of oxidative stress and antioxidant activity to identify possible mechanisms involved in the alterations induced by dexamethasone. Fig. 4 shows the results obtained from the determination of lipid peroxides (LPX), which revealed significant differences between all treatments and the control and DMSO groups. However, no significant differences were observed in MDA production between the treatment groups (20, 40, and 60 ng/L). Moreover, the hydroperoxide content (HPC) showed significant differences between all treatments and the control and DMSO groups, with significant differences between the 20 ng/L and 60 ng/l groups. Similarly, the carbonyl protein content (CPC) exhibited a concentration-dependent increase and statistically significant differences compared to the control and DMSO groups for all treatments. In contrast, the antioxidant activity of SOD was significantly lower than that in the control and DMSO groups. In addition, catalase (CAT) activity was lower in the treated groups, but no significant differences were observed compared to the control groups.

3.3. AChE activity

Fig. 5 shows the AChE activity in the brains of fish exposed to DEXA. After 28 days of dexamethasone exposure at all concentrations, AChE activity decreased significantly compared to that in the control and

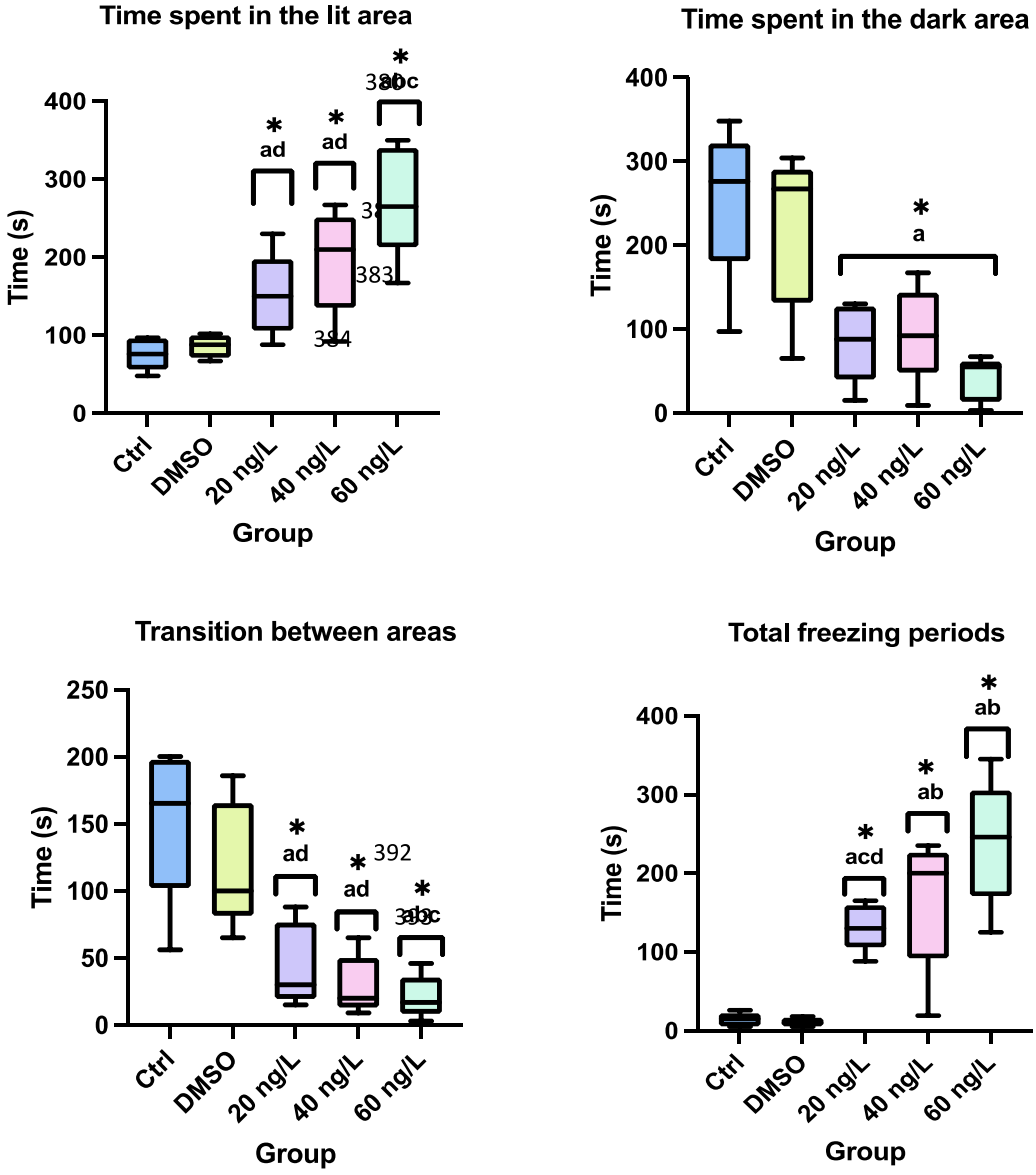

Fig. 3. *D. rerio* adults' behavioral patterns observed in the Light and Dark Test after 28 days of exposure to DEXA. The values represent the mean of three replicates ± SEM. *Significative differences regarding the non-exposed (Ctrl) group. Letters show statistically different values compared to specimens exposed to [a] DMSO, [b]20 ng/L, [c]40 ng/L, [d]60 ng/L. Two-way ANOVA $p<0.05$. Time on the lit side ($F_{(2.82)} = 11.87$; $p<0.05$); Time in the dark side ($F_{(2.82)} = 13.15$; $p<0.05$); Frequency of transitions between sides ($F_{(2.82)} = 17.92$; $p<0.05$); Freezing periods ($F_{(2.82)} = 24.43$; $p<0.05$).

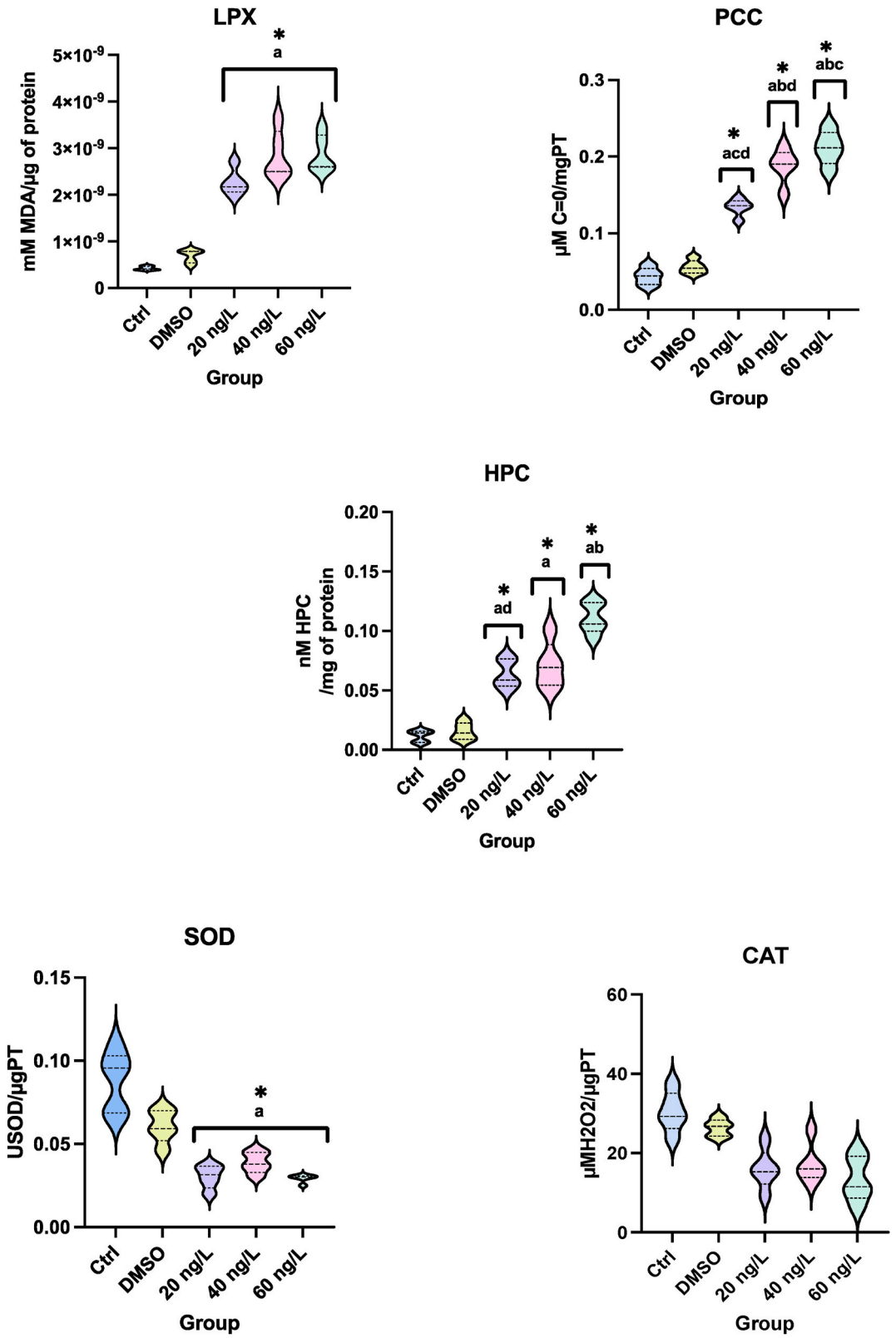

Fig. 4. Biomarkers of cellular oxidation and antioxidant activity evaluated in the brains of *D. rerio* adults induced by DEXA after 28 d of exposure. The values represent the mean of five replicates ±SEM. *Significative differences regarding the non-exposed (Ctrl) group. Letters show statistically different values compared to specimens exposed to [a] DMSO, [b]20 ng/L, [c]40 ng/L, [d]60 ng/L. Two-way ANOVA $p<0.05$. LPX ($F_{(2.82)} = 16.52$; $p < 0.05$); HPC ($F_{(2.82)} = 8.40$; $p < 0.05$); CPC ($F_{(2.82)} = 87.76$; $p < 0.05$); sod ($F_{(2.82)} = 32.43$, $p < 0.05$).

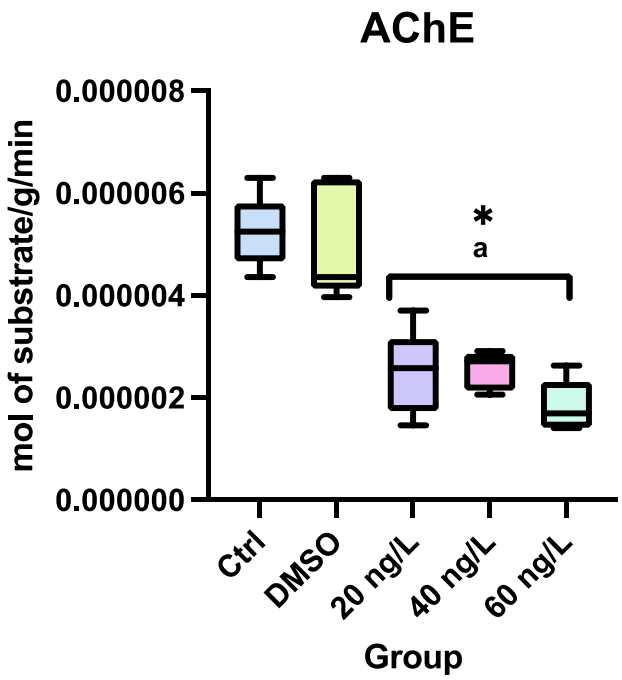

Fig. 5. Graphical representation of the values of AChE enzyme activity evaluated in the brains of *D. rerio* adults after 28 days of exposure to DEXA. The values represent the mean of five replicates ±SEM. *Significative differences regarding the non-exposed (Ctrl) group. Letters show statistically different values compared to specimens exposed to [a] DMSO. Two-way ANOVA $p < 0.05$. AChE activity ($F_{(2.82)} = 32.56$; $p < 0.05$).

DMSO groups; however, there were no significant differences in AChE activity between the treatment groups. The figure depicts substantial disparities between the treatment and control groups.

3.4. Evaluation of gene mRNA expressions of p53, bax, nrf1, nrf2, bcl2 and caspase 3

To determine the mechanism responsible for the developmental toxicity of DEXA, the expression levels of genes related to oxidative stress-induced apoptosis and antioxidant defense were examined (Fig. 6). The *p53* gene, whose activation is generated by apoptotic processes, showed the lowest expression in the 20 ng/L group, with dose-dependent upregulation in the other two treatment groups. On the other hand, the *bcl-2* family, which regulates the release of cytochrome *c* from mitochondria and is considered the primary regulator of apoptosis, had a similar expression to the control and DMSO samples for all treated groups. *bax*, which promotes cell death, was up-regulated in a dose-dependent manner after exposure to all environmental concentrations. *nrf2* is linked to oxidative stress (Chen et al., 2022), had its highest expression in the 60 ng/L group. The levels of the mitochondrion-dependent apoptosis gene *caspase-3* were increased in all treated groups compared to those in the control and DMSO groups. The mRNA expression level of *nrf1*, located in the endoplasmic reticulum participating in the ROS-P53-caspase-dependent apoptotic pathway, was upregulated in all treated groups compared to the control and DMSO samples, with its highest expression after exposure to 40 and 60 ng/L.

Fig. 6. Heat map showing the expression of genes involved in apoptosis (*p53, bcl2, bax, casp3*) and antioxidant responses (*nrf1* and *nrf2*) in *D. rerio* brains after exposure to DEXA (28 days). The data presented illustrate the values ± SEM derived from six separate experiments. ***p53***: [$F_{(2.82)} = 0.214$], ***bcl2*** [$F_{(2.82)} = 0.421$], ***nrf1*** [$F_{(2.82)} = 0.452$], ***nrf2*** [$F_{(2.82)} = 0.219$], ***bax*** [$F_{(2.82)} = 0.543$], ***casp3*** [$F_{(2.82)} = 0.321$].

3.5. Histological alterations

After 28 days of exposure, both the granular cells and molecular layers from the collected brains were observed using microscopy. The study of brain function using tissues involved comparing the treated organisms to a control group. Deterioration occurs when cells cannot maintain their normal function after prolonged exposure (Cambier et al., 2010). These changes appear as microscopic lesions when observed under a microscope. In the zebrafish brains of the non-exposed groups, the typical arrangement of its brain regions was visible through microscopic examination.

The values represent the mean of three replicates ±SEM. *Significative differences regarding the non-exposed (Ctrl) group. Letters show statistically different values compared to specimens exposed to [a] DMSO, [b]20 ng/L, [c]40 ng/L, [d]60 ng/L. Two-way ANOVA, Tukey HSD $p < 0.05$.

In this study, we examined structural alterations in the brains of adult zebrafish following prolonged exposure to dexamethasone. Microscopic analysis showed a normal architecture of diverse areas of the brain with homogenous and well-defined structures (Fig. 7). In Fig. 8, the control group showed a normal structure of the periventricular gray zone, the treated groups showed a thickened or thinned and disrupted granular layer, granule cell loss, cell swelling, pyknotic nuclei,

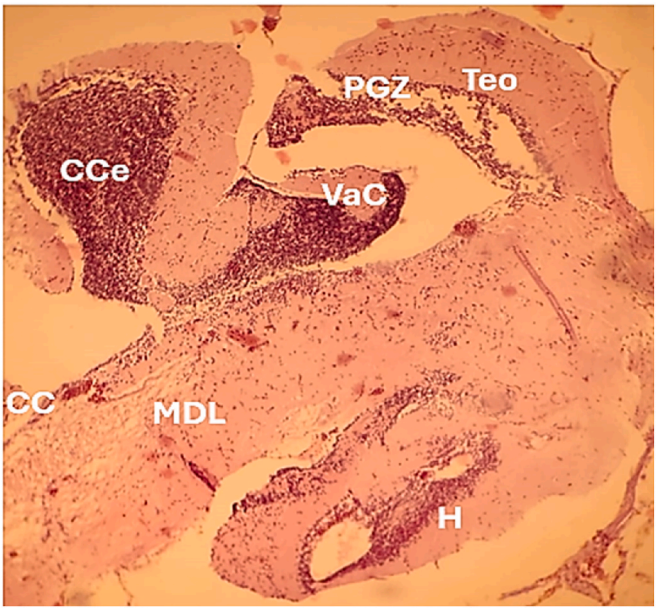

Fig. 7. The microscopic perspective of the brain in the adult zebrafish control group is shown here (H&E staining). PGZ: Periventricular gray zone of the optic tectum; CCe: Corpus cerebelli; H: Hypothalamus; CC: Crista cerebelli; Teo: Tectum opticum; MDL: Medulla; VaC: Vulva cerebelli.

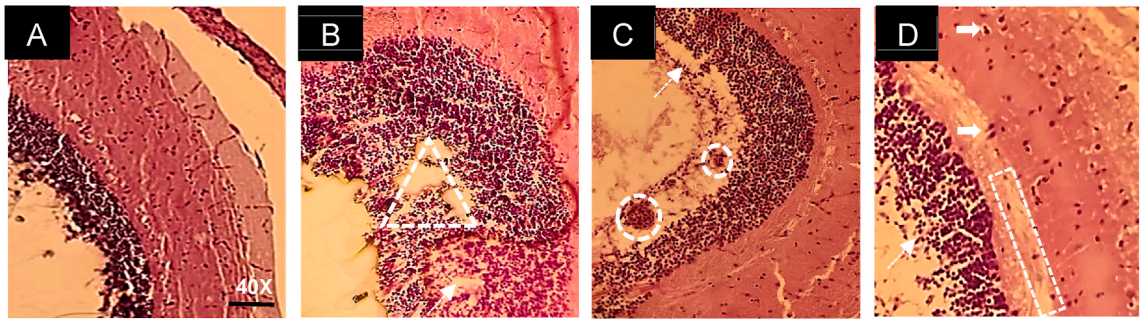

Fig. 8. Microscopic view of the periventricular gray zone (PGZ) of zebrafish exposed to dexamethasone (40x). **A** PGZ of normal zebrafish belonging to the control group, 28 days. **B** PGZ of the group exposed to 20 ng/L**. C** PGZ of the group exposed to 40 ng/L. **D** PGZ of zebrafish exposed to 60 ng/L of DEXA. (Thin arrow) granule cell loss, (thick arrow) cell swelling and pyknotic nuclei, (Rectangle) Intracellular space, (Triangle) disrupted granular layer, (Circle) diffuse congestion. Scale bar shown in (A) applies for all images.

and mild intracellular spaces with diverse widths of the molecular layer in the group exposed to 60 ng/L of DEXA. The optic tectum serves as an essential component of an animal's visual system, as it is responsible for capturing prey and eliciting avoidance behavior. Furthermore, it provides valuable information for navigation purposes. PGZ, a specific region of the optic tectum, plays a vital role in these functions (Abbas et al., 2017).

As shown in Fig. 9, the Ctrl group showed a homogenous structure of the molecular and granular layers of CCe. Cerebellum, is linked to the areas of sensorimotor control, learning and memory processes, and emotional functions. While the treated groups showed a disrupted granular layer with cell swelling and pyknotic nuclei, diffuse congestion, perineuronal vacuolations, and intracellular spaces. The Medulla, a structure that contains neurons critical for cardiovascular, respiratory, metabolic, and motor control of the treated groups showed: intercellular spaces of different width, perineuronal vacuolations, pyknotic cells and diffuse congestion (Fig. 10). The brain medulla, Table 2 illustrates the prevalence of histological alterations in each brain area analyzed per treatment. The data revealed that, in the periventricular zone, treatment with 60 ng/L yielded the highest percentage of alterations (57.14%), significant differences were observed when compared to the unexposed group and between the groups. In contrast, in the corpus cerebelli, treatment with 40 ng/L resulted in the highest number of alterations. Notably, treatment with the highest concentration resulted in the highest percentage (71.4%) of histological alterations in the medulla with significative differences when compared to the unexposed group and between the groups.

3.6. DEXA measurements in water samples and brain tissue

Analysis of brain tissue revealed the presence of DEXA at each of the three tested concentrations in a concentration-dependent manner. Furthermore, consistent bioconcentration factors were observed within the range of 0.64 0.80. These findings suggest a proportional uptake of DEXA by brain tissue, indicating a consistent pattern of bioaccumulation across the different concentrations tested, as demonstrated in Table 3.

3.7. Pearson's correlation analysis

The Pearson correlation analysis (Fig. 11) exhibited a diverse array of positive and negative correlations among behavioral data, oxidative response biomarkers, antioxidant defense, AChE enzymatic activity, and gene expression following all treatments. These results underscore the interconnectedness and reciprocal influence of various biological indicators, thereby illustrating the far-reaching impact of DEXA exposure on the studied physiological and molecular parameters.

4. Discussion

Synthetic glucocorticoids (GCs) are chemicals that have been shown to be present in aquatic environments around the globe, with concentrations in the low nanomolar range (ng/L) (Lindim et al., 2016; Praveena et al., 2018). These compounds are commonly used in medical treatment because of their immunosuppressive, anti-inflammatory, and anti-allergic properties. Researchers are eager to determine whether dexamethasone (DEXA), a highly potent synthetic glucocorticoid, can induce neurotoxic effects in nontarget organisms when exposed to environmentally relevant concentrations. This manuscript emphasizes the outcomes discovered and elucidates the underlying mechanisms responsible for the effects of DXE on the neurobehavior of grown zebrafish. GCs have been shown to play crucial roles in brain development (Jaszczyk and Juszczak, 2021), however, there is consistent evidence that prolonged exposure to GC can have long-lasting effects on the nervous system.

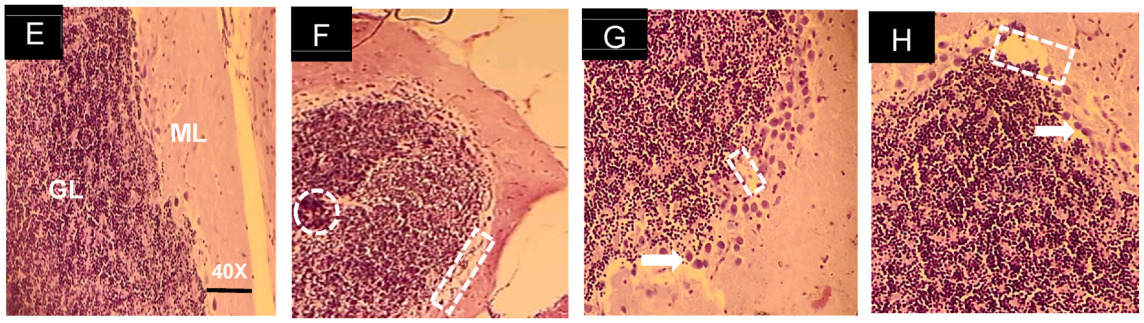

Fig. 9. Microscopic view of the Corpus Cerebella (CCe) of zebrafish exposed to dexamethasone. **E** CCe of normal zebrafish belonging to the control group, 28 days. **F** CCe of the group exposed to 20 ng/L**. G** CCe of the group exposed to 40 ng/L. **H** CCe of zebrafish exposed to 60 ng/L of DEXA. GL: granular layer, ML: molecular layer. (Rectangle) Intracellular space, (thick arrow) cell swelling, (Circle) diffuse congestion. Scale bar shown in (**E**) applies for all images.

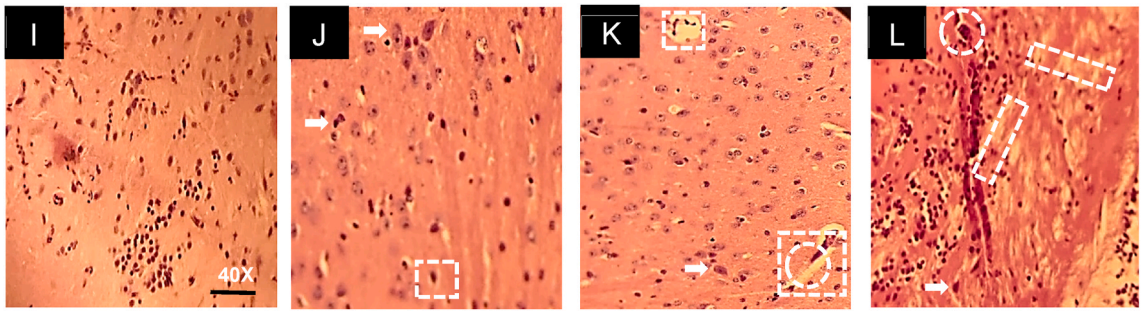

Fig. 10. Microscopic view of the Medulla (MDL) of zebrafish exposed to dexamethasone. **I** MDL of normal zebrafish belonging to the non-exposed group, 28 days. **J** MDL of the group exposed to 20 ng/L. **K** MDL of the group exposed to 40 ng/L. **L** MDL of zebrafish exposed to 60 ng/L of DEXA. (Square) perineuronal vacuolations, (Circle) diffuse congestion, (Rectangle) intracellular space, (Thick arrow) pyknotic nuclei. Scale bar shown in (**I**) applies for all images.

Table 2
Prevalence matrix of alterations found in brain of *D. rerio*.

Brain zone	Treatment	Tissular alterations							% Prevalence
		Intracellular space	Cell swelling	Diffuse congestion	Granule cell loss	Pyknotic nuclei	Disrupted granular layer	Perineuronal vacuolations	
Periventricular gray zone	Ctrl								0%
	DMSO								0%
	20 ng/L				X		X		28.6%^{*,a,d}
	40 ng/L			X	X				28.6%$^{*,a,\ d}$
	60 ng/L	X	X		X	X			57.14%^{*,a,b,c}
Corpus Cerebella	Ctrl								0%
	DMSO								0%
	20 ng/L	X		X					28.6%^{*,a,c}
	40 ng/L	X	X		X				42.9% ^{*,a,b,d}
	60 ng/L	X	X						28.6%^{*,a,c}
Medulla	Ctrl								0%
	DMSO								0%
	20 ng/L					X		X	28.6%^{*,a,c,d}
	40 ng/L			X		X		X	42.9%^{*,a,b,d}
	60 ng/L	X	X	X		X		X	71.4%^{*,a,b,c}

Table 3
Values are the mean of three replicates ± S.D. DEXA LOQ for water samples: 3.42 ng/L ng/L and DEXA LOQ for brain tissue: 0.52 ng/g.

Concentration (nominal) ng/L	DEXA detected in water samples (ng/L)	*DEXA detected in brain samples (ng/g)*	Bioconcentration Factor (BCF)
Ctrl	*ND*	*ND*	***ND***
DMSO	*ND*	*ND*	***ND***
20	9.3	7.5	***0.80***
40	17.6	11.5	***0.65***
60	26.8	17.3	***0.64***

Previous studies have shown that DEXA increases the susceptibility of brain cells to biochemical alterations, leading to behavioral disruptions (Mutsaers and Tofighi, 2012). However, the intracellular mechanisms that underlie this process remain unclear. Behavioral assays are effective, potent, and highly sensitive tools for evaluating the possible detrimental effects of xenobiotics on the nervous system (Kalueff et al., 2013). NTT and LDT are commonly utilized techniques for evaluating parameters such as locomotor activity, anxiety-related behaviors, and stress indicators such as scototaxis, which is a response to chemical or physical disturbances (Fontana et al., 2022).

Our behavioral battery results showed that zebrafish adults experienced an anxiety-like state and stress due to prolonged exposure to DEXA. The NTT exhibited a hypolocomotion-like state, as observed through a decrease in the total distance traveled. This finding is in line with prior research on zebrafish exposed to prednisolone and mice exposed to dexamethasone, both of which showed a reduction in activity and periods of immobility (Conti et al., 2017; Xin et al., 2020). The consequences of a reduced locomotion are less social behavior, feeding, and growth, and hence, reduced fitness (Brodin et al., 2014). Thigmotaxis exhibited a concentration-dependent pattern. Specifically, the time spent in the upper portion of the environment was reduced and there was an increase in the latency to explore that area. In addition, the number of transitions to the upper part decreased, suggesting a prolonged period of immobility or freezing episodes. Evidence suggests that stressed fish exhibit reduced exploration, increased latency to reach the top, prolonged and frequent freezing, and elevated erratic movement (Bai et al., 2016; Collier et al., 2017; Faria et al., 2018). Contrary to our study, Wilson et al. (2016) observed that zebrafish treated with dexamethasone spent more time exploring the upper region of the tank than the control group, based on their results on GC activity, which promoted anxiogenic and anxiolytic responses. In agreement with our study, Golla et al. (2020) reported that chronic exposure to stressors causes a significantly higher proportion of fish to swim at the bottom of the tank. The ability to make choices and undertake appropriate actions is crucial for ensuring the survival and well-being of individuals. The documented behavior of zebrafish shows their ability to exhibit phototaxis, which is characterized by a rapid and transient movement toward or away from a light source and typically lasts for less than 1 min (Lau et al., 2011). In a normal situation, zebrafish adults exhibit light avoidance behavior as a form of survival (scototaxis); however, when individuals are exposed to an external stressor, internal biochemical states or altered motor capabilities play an important role in the lit/dark choice (Maximino et al., 2010). It is worth noting that in the current LDT, the subjects spent

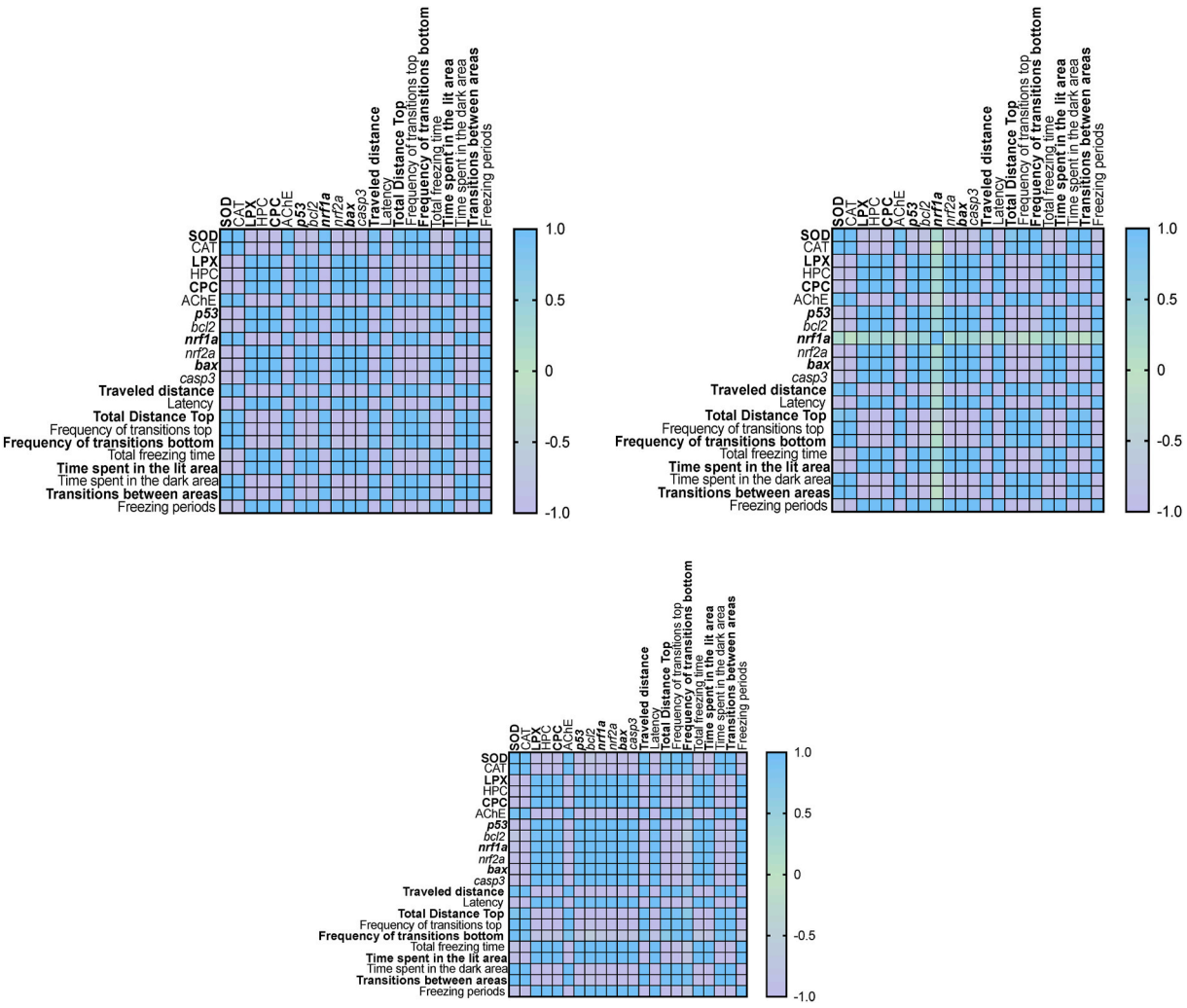

Fig. 11. Heat maps showing Pearson's Correlation matrices for (a) 20 ng/L, (b) 40 ng/L and (c) 60 ng/L.

significantly less time and displayed a higher latency to enter the dark compartment of the testing apparatus than the white compartment. In addition, they moved notably less in the dark. In this particular case, the behavior of the treated individuals in the white compartment suggests a conflict between the desire for safe spaces and the circumstances in the specific area and an inherent drive to investigate new surroundings. Anxiety-related phenotypes that manifest as aversion or avoidance are linked to exposure to stressors (Richendrfer et al., 2012), as seen in this study. This behavioral pattern is known as scotophobia. Research conducted by Champagne et al. (2010) indicated that environmental stressors can lead to the manifestation of anxiety-like behaviors in zebrafish, such as avoidance of dark areas in light/dark box tests, which in turn impacts their exploratory patterns and defensive behavior. In contrast, McNeil et al. (2016) reported that exposure to prednisolone, a synthetic glucocorticoid, increased the preference of zebrafish embryos for dark areas in the LDT. The hypothalamic-pituitary-interrenal (HPI) axis present in teleost fish plays a major role in fight or fly responses as it controls the release of cortisol, which binds to glucocorticoid receptors, resulting in an adaptive mechanism against stress (Zhang et al., 2015). A possible mechanism that explains the behavior shown in this study is expounded by Best et al. (2017) who demonstrated that altered cortisol levels in larval zebrafish, enhanced the "boldness" of the individuals to spend more time in the white compartment than in a darkened safer environment. DEXA, a molecule resembling cortisol, may disrupt this response because of its structural similarity to endogenous hormones. Chronic exposure to chemical stressors may affect the HPI axis as well as the noradrenergic, glutaminergic, dopaminergic, and serotoninergic systems, resulting in diverse behavioral dysregulations (Chrousos, 2009; Popoli et al., 2012; Sandi and Haller, 2015).

As observed in our study, Adedara et al. (2022) attributed anxiety-like behavior to a decrease in AChE activity. The central cholinergic system and its role in regulating behavior through acetylcholine signaling are significant in both humans and animals. For instance, Giacomini et al. (2020) observed a link between the inhibition of AChE activity and an increase in glucocorticoid activity in zebrafish, which contributed to the development of anxiety-like behavior. Acetylcholinesterase (AChE) breaks down acetylcholine in cholinergic synapses, enabling effective communication at the synaptic level. However, when AChE is inhibited, acetylcholine accumulates in the synaptic space, leading to overstimulation of cholinergic receptors, followed by depression, paralysis, and potentially death (Bonansea et al., 2016). It is intriguing to consider the outcomes of behavioral tests and AChE activity, given that hypoactivity and hyperactivity, conduct towards new stimuli, and spatial memory can be connected to the suppression of AChE function (Senger et al., 2011). According to our results on oxidative stress biomarkers, the inhibition of AChE activity may be explained by this mechanism of toxicity. Some studies have indicated that oxidative stress induced by the presence of pharmaceutical compounds may affect AChE activity. For instance, Pullaguri et al. (2020) concluded that oxidative stress was linked to the downregulation of AChE activity in the brain tissue of adult zebrafish exposed to triclosan. Moreover, Ezeoyili et al. (2019) reported a possible correlation between

altered brain oxidative biomarkers and AChE inhibition in African Catfish after acute exposure to sublethal concentrations of carbendazim.

Glucocorticoids play a critical role in maintaining balance and in responding to stress in the nervous system, as reported by Garabedian et al. (2017). These hormones affect the mitochondrial function in two distinct ways. At low concentrations and short-term exposure, they induce biogenesis and enzymatic activity of various complexes, while the long-term effects result in dysfunction of the respiratory chain, generation of reactive oxygen species (ROS), apoptosis, and cell death (Aedo et al., 2019; Duclos et al., 2004; Manoli et al., 2005).

Our study showed that environmental concentrations of DEXA increased the biomarkers of oxidative alterations, such as lipid peroxidation, hydroperoxide content, and protein carbonyls, in association with a decrease in the antioxidant enzyme activities of SOD and CAT as evidenced by biomarkers shown in Fig. 4. These results may be explained as GC have been shown to suppress mitochondrial respiration (Morin et al., 2000) and induce mild mitochondrial depolarization in neural cells as they actively participate in mitochondrial oxidation, loss of the membrane potential and alterations in Ca^{2+} retention (Du et al., 2023). Mitochondrial dysfunction can result from high intracellular calcium levels. Increased glucocorticoid concentrations can cause a sustained calcium influx, leading to ROS production (Guo et al., 2021). This may explain the increase in cellular oxidation biomarkers and the elevated activity of the antioxidant enzymes SOD and CAT observed in this study. Previously, Pes et al. (2022) reported significant alterations in antioxidant activity after exposure to 0.392 mg/L DEXA in *Solea senegalensis*. In addition, Guiloski et al. (2015b) reported an increase in LPO in the livers of male fish *Hoplias malabaricus* after exposure to 0.0–3.0 μg/kg of DEXA.

Mutsaers and Tofighi (2012) suggested that neuronal damage induced by DEXA might be explained by a decrease in the gene expression of mitochondria-encoded respiratory chain enzymes, leading to mitochondrial dysfunction, increased ROS production, and undermined behavioral patterns. GCs bind to both glucocorticoid and mineralocorticoid receptors, resulting in a variety of genomic and non-genomic effects that are crucial for a swift response to stressors but can also contribute to the development of certain illnesses.

Excessive exposure to elevated glucocorticoid levels, such as dexamethasone, triggers neuronal plasticity, leading to structural and functional changes resulting from modified neuronal activity. This is because glucocorticoid receptors are widely dispersed throughout the central nervous system and are activated by high glucocorticoid concentrations (Madalena and Lerch, 2017).

According to Prenek et al. (2017) these compounds exert non-genomic effects by utilizing their phosphorylated receptor form. N-methyl-D-aspartate receptor activity is crucial for neuronal plasticity, and chronic stress can lead to a decrease in synapse number, negatively impacting memory and behavior. Glucocorticoids enter the mitochondria bound to the receptor and activate enzyme complexes that produce reactive oxygen species.

According to previous reports, GCs pretreatment may affect apoptotic cell death in brain tumors by activating the transcription of *bcl-xl*. Apoptosis, also referred to as programmed cell death (PCD), involves a sequence of events, such as the activation of diverse caspases, mitochondrial depolarization (Tang et al., 2023), cellular volume loss (Rana et al., 2020), chromatin condensation (Jeffery et al., 2021), and DNA breakage (Tsuchiya, 2020). The *bcl-2* family of genes plays a crucial role in the regulation of apoptosis (Qian et al., 2022). Members of this family, such as *bcl-2* and *bcl-xl*, promote cell survival by acting as anti-apoptotic proteins, whereas pro-apoptotic members, such as *bax* and *bcl-xs*, facilitate cell death (Kunac et al., 2022). Moreover, it has been reported that the interruption of mitochondrial membrane potential by ROS results in the release of cytochrome *c* and other apoptogenic proteins, ultimately leading to the activation of caspase 9 and effector caspase 3, resulting in the induction of apoptosis. Chen et al. (2020) found that DEX reduced the expression of bcl2 and increased the expression of *bax*, cytochrome C, and *apaf1* in C2C12 cells treated with 500 and 1000 μM for 24 h as observed in this study.

Moreover, in a study performed on osteoblasts, Yun et al. (2009) found that dexamethasone induced apoptosis in examined cells through caspase activation depending on its binding to the glucocorticoid receptor (GR). In accordance with our results, the potential mechanism by which high-dose DEXA exposure predisposes cells to apoptosis induction may involve a reduction in *bcl-2* expression and concomitant increase in *bax* expression (Ghasemi et al., 2018).

Our study found that the tumor suppressor protein p53 showed a concentration-dependent tendency to upregulate expression compared to the control group. In research conducted by Jayamurali and Narayanan Govindarajulu (2017) they examined the consequences of persistent stress on apoptotic gene expression, specifically focusing on p53. These findings suggest that this condition may lead to changes in the apoptotic pathway, including an increase in p53 expression and a decrease in mitochondrial bcl-2, ultimately resulting in negative effects on zebrafish neuronal cells. As mentioned before, several studies have demonstrated that damage to mitochondria can lead to the production of harmful byproducts known as reactive oxygen species (ROS), which in turn promote inflammation and modify gene expression. As previously demonstrated, an increase in ROS levels causes DNA damage. Additionally, the tumor suppressor protein p53 is crucial for regulating DNA damage response at the cellular level. Studies have also revealed that p53 can detect oxidative DNA damage, which may activate p53-dependent apoptosis in the zebrafish brain (Aschbacher et al., 2013; Picard et al., 2014; Zhu et al., 2011).

In addition, we determined the impact of exposure to DEXA on the expression of the transcription factors *nrf1* and *nrf2*, which are known to regulate oxidative phosphorylation, mitochondrial metabolic activities, and response to oxidative stress. In vertebrates, *nrf1* and *nrf2* play pivotal roles in defense against electrophiles. Both genes protect against DNA and protein damage through their ability to combat oxidative stress, thereby reducing the risk of cancer and respiratory or neurodegenerative disorders (Sykiotis and Bohmann, 2010). Our study showed an increase in the expression of both *nrf1* and *nrf2* genes in the treated groups, indicating that the activity of the brain counteracts the oxidative damage induced by dexamethasone. Histopathology effectively visualizes stress-induced structural changes in cells and tissues and is widely used as a biomarker for evaluating various stressors (Bernet et al., 1999). The findings pertaining to the microscopic changes in various brain regions were thoroughly corroborated by modifications in oxidative stress indicators, in addition to the modified behavior of zebrafish and genes expression. The brains of adult zebrafish are quite similar to those of higher animals and are composed of five main regions: the telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon with sub-regions such as periventricular gray zone of the optic tectum, corpus cerebelli, hypothalamus; crista cerebelli, tectum opticum, medulla, vulva cerebelli among others (Patel et al., 2023). Regarding the 28-day exposure to DEXA, we found mild alterations such as perineuronal vacuolations, intracellular spaces, loss of granular cells, deterioration of granular and molecular layers, pyknotic cells, cell swallowing, and diffuse congestion in different zones of the cerebellum, periventricular gray zone, and medulla. The periventricular gray zone in the optical tectum is crucial for the visual system, avoidance movements, feeding, sensory discrimination, and rapid decisions (Kinoshita and Ito, 2006), so structural changes may alter behavioral patterns. Our study corroborates Mishra and Devi (2014) who found that an AChE activity inhibitor affects the PGZ and optical tectum, causing necrosis, congestion, and vacuolation of granular cells, which impaired the visual and mechanical movements of the teleost fish *Channa punctatus*. Additionally, the cerebellum, another region studied in this investigation, is linked to sensorimotor control, learning and memory processes, and emotional functions. Similarly, Rodríguez et al. (2005) found that goldfish with cerebellar damage exhibited severe spatial cognitive impairment and mildly impaired exploratory behavior. In accordance

with our observations Onofre-Camarena et al. (2024) acutely exposed zebrafish adults to dexamethasone finding diverse alterations including: pyknosis, leukocyte infiltration, diffuse congestion, and vacuolization on liver tissue. Moreover, a study performed by Zhong et al. (2021b)also shown that following a 2 month-long exposure to DEXA (0.5–5 μg/L), liver tissue developed vacuolation, dilated hepatocytes, focal necrosis, pyknotic caryon, and other degeneration signs. Finally, Sharif et al. (2015) concluded that long-term exposure to DEXA was responsible to produce immune cells death by both apoptosis and necrosis on zebrafish embryos.

The analytical examination of brain tissue revealed a concentration-dependent occurrence of DEXA, and higher bioconcentration factors were observed for all concentrations that were evaluated (Table 3). This uniform pattern of DEXA uptake by the brain tissue, regardless of the concentration, supports our results.

Based on the findings of this study, we propose a model that elucidates the effect of dexamethasone (DEXA) on adult *D. rerio* brains (Fig. 12, Supplementary Data).

5. Conclusions

Dexamethasone (DEX), a synthetic GC agonist commonly used in both medical and veterinary treatments, possesses anti-inflammatory properties and immunosuppressive activity owing to its interaction with the glucocorticoid receptor (GR) in fish cells (Bury and Sturm, 2007). The worldwide consumption of this pharmaceutical compound has increased due to the COVID-19 pandemic since 2020. According to a report by Polaris Market Research, the market for corticosteroids, including GCs, is predicted to experience a growth rate of >4.6% between 2024 and 2032, leading to a significant increase in environmental occurrence.

Prolonged exposure to DEXA has been shown to trigger a substantial toxic reaction in mammals including atrophy of the hippocampus, cognitive decline, and depression (Glumac et al., 2017; Korewo-Labelle et al., 2023; Mesripour et al., 2019). However, the mechanisms involved in the toxic response in teleost fish have not been well investigated.

Our research demonstrates that prolonged exposure of adult *Danio rerio* to DEXA results in an anxiety-like state, stress, and dark-avoidance behavior as well as a neophobic response within the behavioral battery. This was accompanied by a significant increase in oxidative biomarkers, including LPX, HPC, and PCC. Furthermore, the defensive action of antioxidant enzymes, such as SOD and CAT, was decreased compared to that in the non-exposed groups. This exposure also caused notable changes in biochemical output, particularly in the activity of AChE, a critical enzyme in the synapsis process. Additionally, DXE exposure led to increased expression of genes associated with defense against stress and apoptosis, except for the bcl2 gene expression, which remained like that in the non-exposed groups. Histological observations revealed a decline in the normal structure of important brain regions at all tested concentrations, which may have resulted in the erratic behavior observed in this study.

CRediT authorship contribution statement

Livier M. Sanchez-Aceves: Writing – original draft, Methodology, Formal analysis, Data curation. **Itzayana Pérez-Alvarez:** Software, Data curation. **Diana Belén Onofre-Camarena:** Formal analysis, Data curation. **Verónica Margarita Gutiérrez-Noya:** Software, Investigation, Data curation. **Karina Elisa Rosales-Pérez:** Supervision, Software, Methodology, Investigation, Data curation. **José Manuel Orozco-Hernández:** Visualization, Supervision, Software, Resources. **María Dolores Hernández-Navarro:** Writing – original draft, Visualization, Validation, Supervision, Formal analysis. **Hariz Islas Flores:** Visualization, Validation, Supervision, Software. **Leobardo Manuel Gómez-Olivan:** Writing – review & editing, Writing – original draft, Resources, Project administration, Investigation, Formal analysis, Conceptualization.

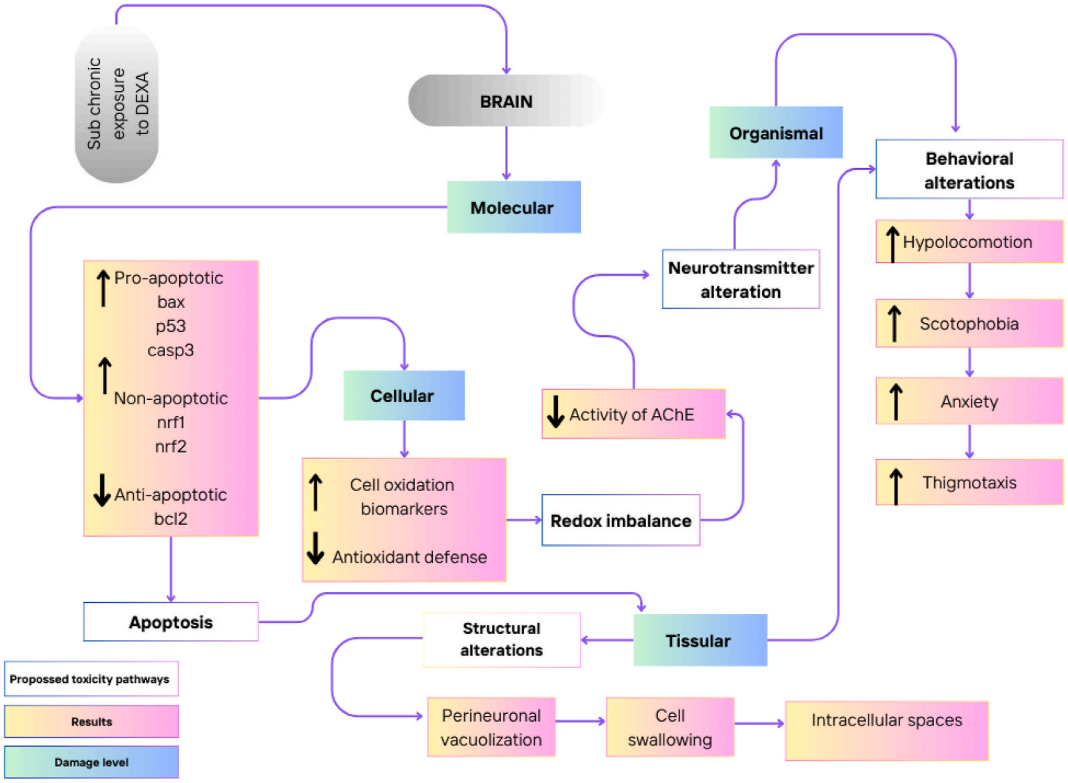

Fig. 12. Flow diagram of results and proposed toxicity pathways.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143012.

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