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RESEARCH ARTICLE

Ochratoxin A induces locomotor impairment and oxidative imbalance in adult zebrafish

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Abstract

Ochratoxin A (OTA) is a mycotoxin produced by species of filamentous fungi widely found as a contaminant in food and with high toxic potential. Studies have shown that this toxin causes kidney and liver damage; however, data on the central nervous system effects of exposure to OTA are still scarce. Thus, this study aimed to investigate the effects of exposure to OTA on behavioral and neurochemical parameters in adult zebrafish. The animals were treated with different doses of OTA (1.38, 2.77, and 5.53 mg/kg) with intraperitoneal injections and submitted to behavioral evaluations in the open tank and social interaction tests. Subsequently, they were euthanized, and the brains were used to assess markers associated with oxidative status. In the open tank test, OTA altered distance traveled, absolute turn angle, mean speed, and freezing time. However, no significant effects were observed in the social interaction test. Moreover, OTA also increased glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR) levels and decreased non-protein thiols (NPSH) levels in the zebrafish brain. This study showed that OTA can affect behavior and neurochemical levels in zebrafish.

Keywords Toxin · Environmental contaminant · Toxicology · Oxidative status · Behavior · Zebrafish

Introduction

It is estimated that 200 thousand people are added daily to the world's demand for food (Nellemann et al. 2009). With the projections that by 2050 the world will reach 9.8 billion inhabitants (United Nations 2017), the search for solutions to meet those needs becomes urgent. Currently, the tools used to solve this issue are responsible for creating other problems. For example, the increase in pesticide use in large crops is already causing serious environmental and public health impacts (World Health Organization 2006; Langley and Mort 2012; Rani et al. 2021). Improperly tampering with livestock products may put consumers' lives in danger (Xin and Stone 2008; Cavin et al. 2018), and industry investment in processed foods has been linked to the incidence of obesity, diabetes, celiac disease, and heart disease (Canella et al. 2014; Anand et al. 2015; Aguayo-Patrón and Calderón de la Barca 2017). Although for a long time environmental conditions and inadequate storage of food products have been ignored, today it is already clear that these conducts are responsible for the increasing presence of mycotoxins (Marroquín-Cardona et al. 2014).

Mycotoxins are naturally occurring compounds in species of fungi and are potentially toxic (Tola and Kebede 2016). Ochratoxin A (OTA) is a mycotoxin produced by filamentous fungi and belongs to the ochratoxin subgroup, along with ochratoxins B and C. However, OTA has more natural occurrence and higher toxicity than other ochratoxins. OTA has become a very common contaminant in food and the ecosystem. There is evidence of the presence of OTA in water sources (Mata et al. 2015; Hu et al. 2017) and sea animals (Sun et al. 2015). The highest incidence of detection, however, occurs in food. OTA has already been found in many types of food in the world, including meats found in Croatia (Pleadin et al. 2015),

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Brazilian and European coffee (v. d. Stegen et al. 1997; de Almeida et al. 2007), wines and beers from Chile and Hungary (Vega et al. 2012; Varga et al. 2014), fruits in Argentina and Canada (Magnoli et al. 2004; Lombaert et al. 2004), European juices (Jørgensen 2005), and several other types of products across the globe. The contamination of feed commodities also affects the preparation of animal feeds with the mycotoxin having already been identified in fish and poultry feed (Abidin et al. 2017; Pietsch et al. 2020; Nogueira et al. 2020).

The exportation market moves around billions of dollars per year (Food and Agriculture Organization (FAO) 2019a) and billions of food tons (Food and Agriculture Organization (FAO) 2019b) are transported to countries with varying laws and cultures. Most nations have protocols and specific regulations for the tolerable limits of contaminants in the food, including OTA (Official Journal of the European Union 2006; Bureau of Chemical Safety et al. 2009; Ministério da Saúde and Agência Nacional de Vigilância Sanitária 2011). However, there is no consensus on acceptable limits for this contaminant among countries and, as the trade develops, the OTA present in food crosses borders and easily spreads around the world due to a lack of consent between health inspection standards.

The mechanism of OTA toxicity is not clear yet. It is believed to be related to the inhibition of protein synthesis caused by the competition between the phenylalanine group of OTA and phenylalanine amino acid (Kőszegi and Poór 2016). The effects of OTA have already been evaluated in rodents (Kanisawa and Suzuki 1978; Castegnaro et al. 1998), birds (Stoev 2010), and fish (Doster et al. 1974; Manning et al. 2003). The toxin has been associated with immune modulation (Lea et al. 1989), hepatic (Qi et al. 2015), and kidney diseases (Abid et al. 2003; Fuchs and Peraica 2005). OTA has also been increasingly associated with neuropsychiatric disorders (Sava et al. 2006a, b; Yoon et al. 2009; Brewer et al. 2013). However, despite the importance of these reports, there is still little information regarding the behavioral and neurochemical effects related to OTA on non-target organisms. Therefore, OTA is an important contaminant for both environment and food commodities, but there are still several gaps in the knowledge about the effects of this toxin in organisms.

Native from Asia, zebrafish is a teleost that has high genetic and physiological homology with humans (Lieschke and Currie 2007). For this reason, this species has been used as a research animal model for different fields such as embryology and development (Keller et al. 2008; Hao et al. 2013), oxidative stress (Choi et al. 2010; Marcon et al. 2018), behavior (Abozaid et al. 2020; Reis et al. 2020; Nabinger et al. 2021), and genetics (Nasevicius and Ekker 2000; Pimentel Falcão et al. 2021). Moreover, this aquatic animal is a very interesting environmental bioindicator used in toxicology and ecotoxicology research due to its capacity to simulate the conditions of an animal in its natural ecosystem (Asharani et al. 2008; Valadas et al. 2019; Park et al. 2020). In this context, since zebrafish is a suitable environmental bioindicator used in toxicology research, this study aimed to investigate the behavior and neurochemical effects of OTA in adult zebrafish.

Materials and methods

Animals

The experiments were performed using 96 adult (4–6-month-old) short-fin wild-type zebrafish (*Danio rerio*, Hamilton, 1822) obtained from a local commercial supplier. Animals were of both sexes (50:50 male:female ratio) with the detailed ratio per group presented in Online Resource 1.

The animals were housed in a maximum density of two fish per liter of water in 16-L tanks (40 × 20 × 24 cm) and under a 14–10-h day/night cycle for 10 days before any procedure. Water parameters such as pH (7.0 ± 0.3), chlorine, ammonia (< 0.01 mg/L), and temperature (26 °C ± 2) were controlled. Fish were fed twice a day with commercial flake food (Poytara®, Brazil) and supplementation of brine shrimp (*Artemia salina*). After the behavioral tests, the animals were euthanized by hypothermic shock (2–4 °C) followed by decapitation, according to the AVMA Guidelines for the Euthanasia of Animals (Leary and Johnson, 2020). All procedures were approved by the Universidade Federal do Rio Grande do Sul ethical committee (#37761/2020). The protocols were reported following ARRIVE Guidelines 2.0 (Percie du Sert et al. 2020).

Chemicals

Ochratoxin A (OTA) (CAS 303-47-9), dimethyl sulfoxide (DMSO) (CAS 67-68-5), and tricaine (MS-222) (CAS 886–86-2) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride solution 0.9% (saline, ADV Farma, SP, Brazil) was obtained from a local commercial supplier. OTA was dissolved into DMSO (final concentration of 10% DMSO). The OTA doses were based on the LD50 for intraperitoneal injection on rainbow trout (*Salmo gairdneri* or *Oncorhynchus mykiss*) (Doster et al. 1972) of 5.53 mg/kg since there are no similar studies on adult zebrafish.

Experimental procedures

After the period of acclimation to the laboratory environment, the animals were divided into the following experimental groups: control (CTRL), 10% DMSO, OTA (1.38, 2.77, and 5.53 mg/kg). Allocation to experimental groups followed randomization procedures with a computerized random number generator (random.org) and the procedure was performed by researchers blinded to the experimental group. The drugs for each experimental group were administered at the beginning of the experiment (at 0 h) by

intraperitoneal injections and the control group received saline. Briefly, the intraperitoneal injections were performed using a Hamilton Microliter™ Syringe (701 N 10 µL SYR 26 s/2″/2) × Epidurakatheter 0.45 × 0.85 mm (Perifix® Katheter, Braun, Germany) × Gingival Needle 30G/0.3 × 21 mm (GN Injecta, SP, Brazil). The injection volume was 1 µL/100 mg of animal weight. The animals were previously anesthetized by immersion in a solution of tricaine (300 mg/L) until loss of motor coordination and reduced respiratory rate. After the anesthesia, the animals were placed in a sponge soaked in water exposing the abdomen and the needle was gently inserted parallel to the spine in the abdomen's midline posterior to the pectoral fins. This procedure was conducted in approximately 10 s (Fig. 1A) (Bertelli et al. 2021).

Following drug administration, the fish were kept in 4-L static tanks (17 × 17 × 17 cm) with two tanks for each concentration to minimize potential tank effects and remained there for 96 h. After 96 h of exposure, the animals were individually submitted to the open tank test (OTT). After this, the animals returned to the experimental tank and remained for 24 h. Then, the animals were submitted to the social interaction test (SIT). Immediately after the SIT, the animals were euthanized, and the brains were dissected and homogenized for the neurochemical assays of the parameters associated with oxidative status. The neurochemical parameter analyses were as follows: thiobarbituric acid reactive substance (TBARS), non-protein thiol (NPSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione reductase (GR). The sex of the animals was confirmed after euthanasia by dissecting and analyzing the gonads.

Open tank test (OTT)

The OTT consists of a white circular arena (24 cm diameter, 8 cm height, and 2 cm water level). In this test, the animals were placed in the center of the arena and the behavior was individually recorded for 10 min (Fig. 1B). The videos were obtained from an upper view and for the analyses, the arena was virtually divided into two zones: center and periphery (Benvenutti et al. 2020). The following parameters were quantified using ANY-Maze software (Stoelting Co., USA): distance, crossings, absolute turn angle, mean speed, freezing episodes, and freezing duration.

Social interaction test (SIT)

In the SIT, fish were placed individually in a central tank (30 × 10 × 15 cm) flanked by two identical tanks (15 × 10 × 13 cm) and filmed from a frontal view for 7 min (Fig. 1C). One of the two tanks positioned beside the central tank (test tank) contained only water (neutral stimulus), and the other contained 10 zebrafish (social stimulus). All tanks were filled with water at a level of 10 cm and in the same conditions. The side of the social stimulus tank was counterbalanced to avoid any eventual bias (Benvenutti et al. 2020). The analyses were carried out with the aid of the ANY-Maze software (Stoelting Co., USA), with the test tank virtually divided into three equal vertical zones (interaction, middle, and neutral). The interaction zone was considered to be next to the tank that contained the social stimulus, while the neutral zone was considered to be next to the neutral stimulus. Animals were placed in the middle zone and had 2 min to habituate to the tank test. After this, the behavior was analyzed for 5 min. The parameters quantified were distance traveled, number of crossings, and time in the interaction zone.

Neurochemical analysis

Following the behavioral tests, the animals were euthanized by hypothermic shock (2–4 °C) and decapitation. The brain samples were then collected to evaluate the oxidative status (Fig. 1D). For each independent sample, four brains were collected right after the euthanasia, pooled, and homogenized in 600 µL of phosphate-buffered saline (PBS). The mixture was centrifuged at 3000 g at 4 °C in a cooling centrifuge and collected the supernatant, which was kept in microtubes on ice until the assays were performed. The protein was quantified according to the Coomassie blue method using bovine serum albumin.

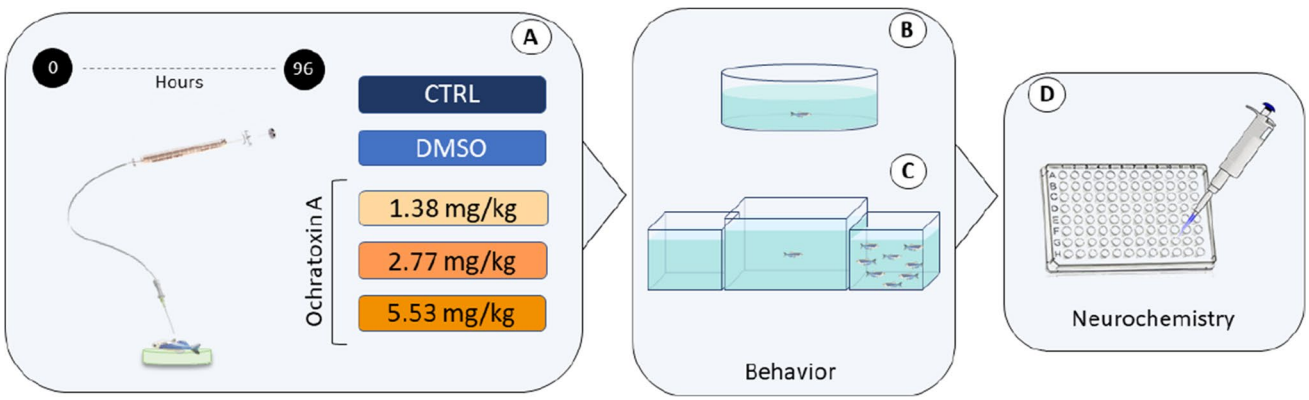

Fig. 1 Experimental design

Thiobarbituric acid reactive substances (TBARS)

The lipid peroxidation was evaluated by analyses of the production of TBARS. Samples (50 µg of proteins) were mixed with thiobarbituric acid (TBA, 0.5%) and trichloroacetic acid (TCA, 20%). The mixture was heated at 100 °C for 30 min. The absorbance was determined at 532 nm in a microplate reader. Malondialdehyde (MDA, 2 mM) was the standard. The detailed protocol is available at Sachett et al. (2020a).

Non-protein thiols (NPSH)

The quantity of NPSH in the samples was determined by mixing the brain tissue preparation (50 µg of proteins) and trichloroacetic acid (TCA, 6%). Then, it was centrifugated (10,000 g, 10 min at 4° C), and the supernatants were added to potassium phosphate buffer (TFK, 1 M). After that, the mixture was added to 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, 10 mM) and the absorbance of 5-thio-2-nitrobenzoic acid (TNB) formed was analyzed at 412 nm after 1 h. The detailed protocol is available at Sachett et al. (2020b).

Glutathione peroxidase activity (GPx)

The GPx levels were determined by a two-step method involving (I) neutralization of hydrogen peroxide (H_2O_2) by GPx in the presence of glutathione reduced (GSH) and (II) recycling of resulting glutathione oxidized (GSSG) by glutathione reductase (GR) in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). For the determination, the sample (30 µg of protein) was mixed with a reaction medium containing TFK + ethylenediaminetetraacetic acid (EDTA) (0.5 M, pH 7.0), NADPH (1.6 mM), GSH (10 mM), GR (2.5 U/mL), and 10 mM azide. Then, H_2O_2 (4 mM) was added and the decrease of NADPH absorbance per minute was read at 340 nm. The detailed protocol is available at Sachett et al. (2021a).

Glutathione reductase activity (GR)

The GR levels were determined as the second part of the aforementioned GPx assay. The sample (30 µg of protein) was mixed with a reaction medium containing TFK + EDTA (154 mM, pH 7.0) and NADPH (2 mM). Then, oxidized glutathione (GSSG, 20 mM) was added and the decrease of NADPH absorbance per minute was read at 340 nm. The detailed protocol is available at Sachett et al. (2021b).

Glutathione-s-transferase activity (GST)

The GST levels were determined by the conjugating reaction of 1-chloro-2,4-dinitrobenzene (CDNB) with GSH GST-mediated (Habig and Jakoby 1981). The sample (30 µg of protein) was mixed with a reaction medium containing 230 µL of TFK + EDTA (100 mM, pH 6.5) and 10 µL of GSH (75 mM). After that, 10 µL of CDNB (30 mM) dissolved in ethanol 95% was added and the increase of absorbance per minute was read at 340 nm.

Statistical analysis

The sample size was calculated using G*Power 3.1.9.2 for Windows. Normality and homogeneity of variances were confirmed for all datasets using D'Agostino-Pearson and Levene tests, respectively. Student's *t*-test was performed to compare the control and DMSO groups. One-way ANOVA followed by Tukey's post hoc test was used for the analyses. For behavioral data, the outliers were identified based on distance traveled using the ROUT statistical test (GraphPad® software) and were removed from the analyses. This resulted in 3 outliers (2 animals from the DMSO group and 1 animal from OTA 2.77 mg/kg group) removed from the OTT and 3 outliers (1 animal from the DMSO group, 1 from the 2.77 mg/kg group, and 1 from the 5.53 mg/kg group) removed from the SIT. The tank and sex effects were tested in all comparisons, and no significant differences were observed. The data were expressed as mean ± standard deviation (S.D.). Differences were considered significant at $p<0.05$.

Results

DMSO did not show important modulation on behavior (Online Resource 2) or oxidative damage (Online Resource 3) compared with sodium chloride control. Therefore, we only used DMSO as a control group.

Open tank test

Figure 2 shows the acute effects of OTA in adult zebrafish in the open tank test. There was a significant decrease in the distance (Fig. 2A, $p = 0.0105$), absolute turn angle (Fig. 2C, $p = 0.0090$), mean speed (Fig. 2D, $p = 0.0110$), and an increase in freezing time (Fig. 2F, $p = 0.0052$) at the 1.38 mg/kg dose, indicating locomotor impairment. The parameters of crossings and freezing episodes were not altered by any dose.

Social interaction test

Figure 3 shows the acute effects of OTA on adult zebrafish at the SIT. OTA, in the tested doses, did not alter social behavior in any of the analyzed parameters.

Neurochemical analysis

Figure 4 shows the effects of OTA on neurochemical parameters. OTA at 1.38 mg/kg increased the GPx (Fig. 4C, $p < 0.0001$), GST (Fig. 4D, $p < 0.0001$), and GR (Fig. 4E, $p = 0.0397$) levels. The intermediate dose of 2.77 mg/kg decreased NPSH levels (Fig. 4B, $p = 0.0006$) and increased GPx (Fig. 4C, $p = 0.0016$) and GST

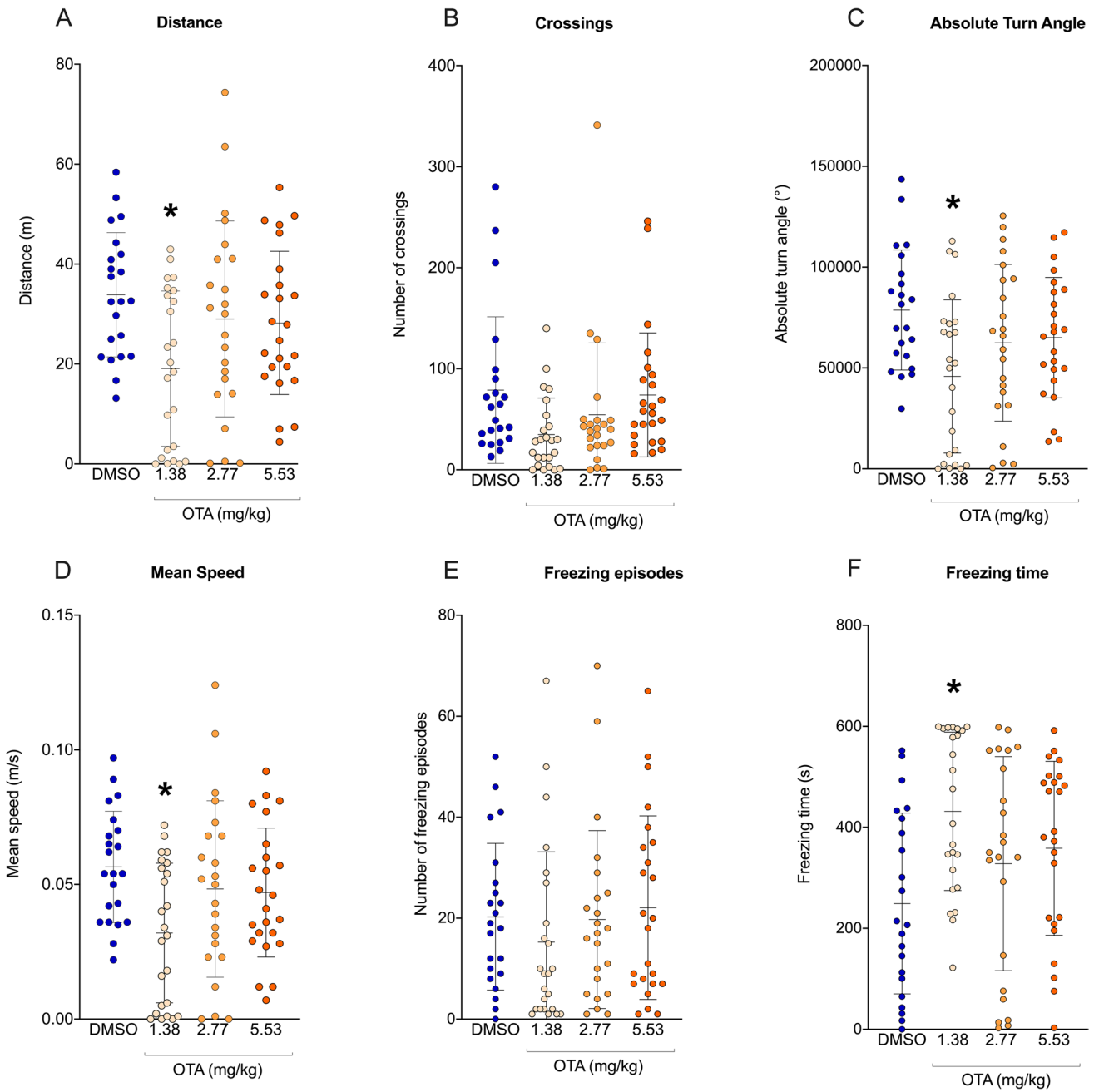

Fig. 2 Effects of OTA in the open tank test. (**A**) Distance, (**B**) crossings, (**C**) absolute turn angle, (**D**) mean speed, (**E**) freezing episodes, and (**F**) freezing time. Data are expressed as mean ± standard deviation (S.D.). n=22–24. One-way ANOVA followed by Tukey's post hoc test. *$p < 0.05$

(Fig. 4D, $p = 0.0146$) levels. The dose of 5.53 mg/kg increased GPx (Fig. 4C, $p < 0.0001$) and GR (Fig. 4E, $p = 0.0238$) levels.

Discussion

This study showed the deleterious effects of ochratoxin A in adult zebrafish. Briefly, the toxin decreased the total distance traveled, average speed, absolute turn angle, and increased the freezing time. However, in the social interaction test, there were no behavioral changes in the evaluated parameters. Neurochemical analysis showed that the compound was able to alter the oxidative status by triggering the oxidative defense system without damage as measured by TBARS.

In zebrafish, OTA has been studied with different emphases, especially with larvae and embryo models (Juan-García et al. 2020). Increased mortality due to malformations has been seen (Csenki et al. 2019). There was a negative effect

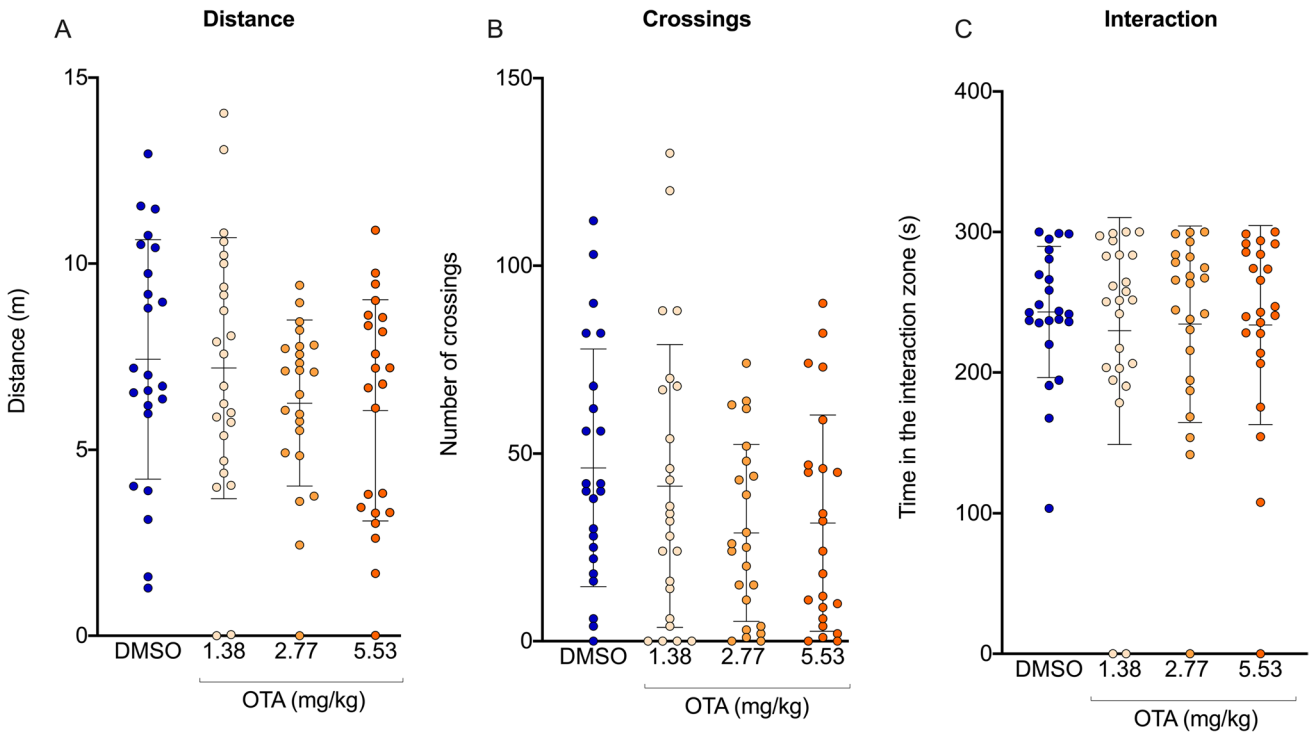

Fig. 3 Effects of OTA in the social interaction test. (**A**) distance, (**B**) crossings, (**C**) interaction time. Data are expressed as mean ± standard deviation (S.D.). *n*=23–24. One-way ANOVA

on the formation of the hepatic and coagulation systems (Wu et al. 2018), occurrence of intracerebral hemorrhage (Wu et al. 2020), and genetic alteration related to nephrotoxicity in a study with OTA individually (Wu et al. 2016) and another that combined OTA with other mycotoxins (Csenki et al. 2021). In other fish species, intestinal disruptions were also seen (Liu et al. 2020), in addition to cell damage and immunosuppression (Zhao et al. 2022).

Although the toxin has been studied before, there are little data in the literature on the behavioral effects of OTA exposure not only in fish but also extending to other animals. In zebrafish larvae, OTA decreased the animals' swimming speed but did not change parameters of distance and time spent active (Khezri et al. 2018). In marine water-reared sea bass (*Dicentrarchus labrax* L.), slow movement, loss of balance, and rapid movement of the operculum as respiratory manifestations were seen (El-Sayed et al. 2009). In what concerns behavior, another study investigated the toxic effects of OTA on Nile tilapia (Oreochromis niloticus) and showed sluggish swimming in the animals and off food patterns (Diab et al. 2018). With rodents, it was shown that OTA injected intraperitoneally was able to cause behavioral changes in gait analysis, spontaneous activity, cylinder test, and pole test, similar to Parkinsonian symptoms that were stabilized with the use of L-dopa (Bhat et al. 2018). In our study, the interference of OTA on locomotion parameters in zebrafish was shown in the open tank test similar to the results previously cited in other models. A possibility for these findings could be the link between locomotion and the nigrostriatal pathway that has already been reported to be affected by OTA in rodent models (Sava et al. 2006a, b). However, there were no changes in social interaction parameters. The social behavior in zebrafish presents a schooling cohesion that aims to search for food, escape from predators, and reproduce (Pitcher 1993). Thus, being a model closely linked to social functions, the zebrafish has been extensively studied for this type of behavior (Buske and Gerlai 2011; Scerbina et al. 2012; Dreosti et al. 2015). However, precisely because socialization is genetically preserved and has an ontogenic nature in zebrafish, it may be a parameter less vulnerable to milder modulations such as those shown in this study, since the lowest concentration used was about 25% of the LD50 established in another species. Another aspect to be considered is related to the cues provided by the apparatus since previous studies have already demonstrated the multifactorial character of social behavior in zebrafish being linked to visual cues (Engeszer et al. 2007), olfactory cues (Gerlach et al. 2007), and also sensitive to alarm substances released by co-specifics (Canzian et al. 2017). The apparatus used in this study, however, only allowed the visual cues to be transmitted to the animal, so it is uncertain to say what the effects of OTA would be under other parameters involved in the animal's social behavior.

With increasing global concern about the spread of mycotoxins, the effect of these compounds on oxidative stress parameters has become a very debated issue (da Silva et al.

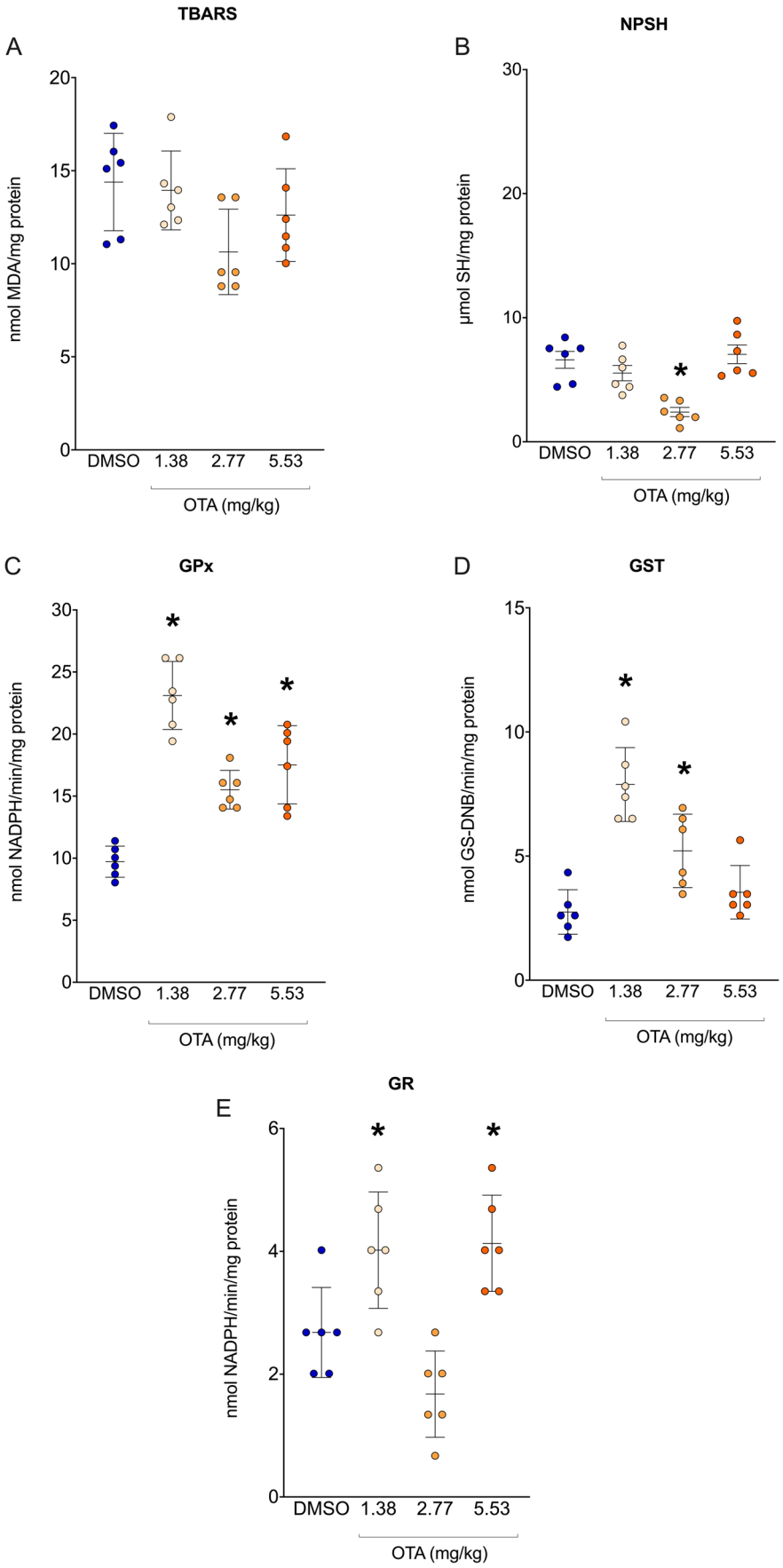

Fig. 4 Effects of OTA in neurochemical parameters. (**A**) TBARS, (**B**) NPSH, (**C**) GPx, (**D**) GST, and (**E**) GR. Data are expressed as mean ± standard deviation (S.D.). n= 6. One-way ANOVA followed by Tukey's post hoc test. *$p < 0.05$

2018; Mavrommatis et al. 2021), with emphasis on ochratoxin A (Sorrenti et al. 2013; Tao et al. 2018). OTA can interact with peroxidases that produce a phenoxyl radical from OTA. Glutathione (GSH) is capable of turning the phenoxyl radical into OTA again by forming a superoxide anion radical ($O_2^{\bullet -}$) that results in hydrogen peroxide (H_2O_2). H_2O_2 by Fenton reaction produces a hydroxyl radical ($OH^{\bullet}$) that is responsible for oxidative damage (Adlouni et al. 2000). Another common pathway for OTA is the formation of an OTA–Fe^{3+} complex that is reduced in OTA–Fe^{2+} by cytochrome P450 resulting in $OH^{\bullet}$ (Rahimtula et al. 1988). Several studies report the imbalance of oxidative status caused by the compound. In zebrafish larvae, there was the formation of reactive oxygen species (ROS) proportional to the increase in OTA concentration (Tschirren et al. 2018). A study with tambaqui (*Colossoma macropomum*), a freshwater fish, found an increase in the ROS and lipid peroxidation in the animal's muscles, as well as a decrease in the levels of antioxidant enzymes superoxide dismutase (SOD) and GPx (Baldissera et al. 2020). Similarly, an increase in lipid peroxidation and antioxidant enzymes activity catalase (CAT) and GR was seen with a decrease in SOD activity and GSH levels in the brain, kidney, and liver of rats (Nogaim et al. 2020). A study found an increase in ROS formation, lipid peroxidation, and decreased GSH levels in kidney cells (Lee et al. 2018). However, studies with birds have shown that in long-term exposure antioxidant defenses can increase against oxidative imbalance, especially the glutathione redox system (Kövesi et al. 2019; Fernye et al. 2021). Also, a study with *Caenorhabditis elegans* showed an increase in the expression of SOD and CAT in wine containing OTA (Schmidt et al. 2020). These studies corroborate with our results which showed that, in adult zebrafish, there was an increase in enzyme defenses with an elevation of GPx, GR, and GST, especially at the lowest dose. In the intermediate dose, there was no increase in GR as occurred in the other doses, which is consistent with the decrease in GSH levels (NPSH) in this group since GR is responsible for the recycling of glutathione, which is essential for the maintenance of antioxidant levels. The increase in GPx under these conditions indicates an attempt to control a possible increase in reactive oxygen species since GPx reduces H_2O_2 through the GSH oxidation, something quite common to occur in OTA exposures as mentioned in previous studies. The increase in GST levels also indicated an increase in OTA metabolization and elimination since GST catalyzes the conjugation of the reduced form of glutathione to xenobiotic substrates for detoxification. Likewise, this activation of defenses prevented the increase of ROS levels and consequently avoiding lipid peroxidation (TBARS levels) (Gandhi and Abramov 2012; Dasuri et al. 2013).

Despite the zebrafish being a model used for decades in research in several areas, many gaps still exist in the model, especially in the area of toxicology. In recent years there has been a considerable increase in studies in this field due to initiatives to standardize this type of analysis in fish (Gonçalves et al. 2020), including the OECD protocols (OECD Guidelines for the Testing of Chemicals 1992). However, for adult animals, the methodologies tend to be limited to direct exposure to the animals' water, which is not suitable for all protocols. In the case of OTA, the formulation of the compound and the difficulty in storing or disposing of waste made this type of exposure impracticable so the intraperitoneal injection standardized in the laboratory was chosen. The use of intraperitoneal injection to assess the effects of OTA has already been used in other models, being effective in detecting deleterious effects on the metabolism mechanism in rats (Størmer et al. 1985), on the immune system (Prior and Sisodia 1982) and neurotoxicity in mice (Miki et al. 1994; Tamaru et al. 1988). In fish, OTA was injected peritoneally into rainbow trout (*Salmo gairdneri*) acutely (96 h) for toxicological evaluation by histology and determination of LD50 (5.53 mg/kg) (Doster et al. 1974). However, these data were never detailed in other species and the use of zebrafish to evaluate the effects of OTA remained limited with little information regarding the effects of the toxin in this species.

The large distribution of OTA among products also makes the toxin's presence in the environment very important. Sun et al. determined a range of 0.0005 mg/kg to 0.0019 mg/kg of OTA found in fish, which is way below the doses used in this study. However, the main focus of OTA contamination is food, specialty cereals, wine, and coffee (Li et al. 2021). (Gruber-Dorninger et al. 2019) determined a large range of OTA on food and its maximum value was 2 mg/kg on cereals. The wide contamination of OTA allows many different dose ranges according to the commodities and in this context, the doses of this study (1.38 mg/kg, 2.77 mg/kg, and 5.53 mg/kg) are relevant to that matter, and the following previous findings.

Due to these important gaps in the literature, another point to be clarified is the dose-response reaction of zebrafish against OTA. In this study, the doses that were more behaviorally and neurochemically reactive were the lowest doses, with the highest dose changing a few parameters in oxidative status. Thus, in this study, we speculate that OTA showed a hormetic effect in adult zebrafish. Hormesis is a biphasic dose-response characterized by stimulation at low doses and inhibition at high doses (Calabrese and Baldwin 2002). For OTA, this type of curve has already been reported in an in vitro study (Li et al. 2014), however, this is the first time that this behavior has been seen in an in vivo model. A biphasic curve can indicate the biological plasticity of the target organism (Calabrese and Mattson 2011), and the zebrafish is a widely studied model precisely because of its

capacity for neuroplasticity and regeneration (Cosacak et al. 2015; Ghosh and Hui 2016). Thus, it is possible that the hormetic behavior of OTA, in this case, is linked to the animal's biological characteristics. Moreover, hormetic curves often occur with endocrine disruptors (Vandenberg et al. 2012) and other studies have demonstrated the potential of OTA to interfere with hormone production (Frizzell et al. 2013; Woo et al. 2013). For all these reasons, toxicological results for low doses should not be ignored.

Conclusion

Although concern about controlling OTA levels is increasing, more efforts are still needed. For this, understanding the effects of the toxin on organisms is essential. This study demonstrated the potential that the toxin has for causing deleterious effects in adult zebrafish through behavioral changes by locomotion impairment and neurochemical modulation of oxidative stress components; however, more studies are needed to elucidate the compound's mechanism of action and its effects on other organisms to further contribute to the field of toxicology and environment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11356-022-23692-4.

Data availability The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Author contribution All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conceptualization: Jéssica Valadas and Angelo Piato; methodology: Jéssica Valadas, Adrieli Sachett, Matheus Marcon, Leonardo M. Bastos, Angelo Piato; investigation: Jéssica Valadas, Adrieli Sachett, Matheus Marcon, Leonardo M. Bastos; formal analysis Jéssica Valadas, Adrieli Sachett, Matheus Marcon, Leonardo M. Bastos, Angelo Piato; resources: Angelo Piato; writing—original draft: Jéssica Valadas; writing—review and editing: Jéssica Valadas, Adrieli Sachett, Matheus Marcon, Leonardo M. Bastos, Angelo Piato; supervision: Angelo Piato; funding acquisition: Angelo Piato. All authors read and approved the final manuscript.

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Declarations

Ethics approval All procedures were approved by the Universidade Federal do Rio Grande do Sul ethical committee (#37761/2020) and were performed following relevant guidelines on the care and use of laboratory animals and following the Brazilian legislation regarding animal research.

Consent to participate Not applicable

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Competing interests The authors declare no competing interests.

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