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INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
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EFEITOS DA EXPOSIÇÃO À OCRATOXINA A SOBRE PARÂMETROS COMPORTAMENTAIS E BIOQUÍMICOS EM PEIXES-ZEBRA (*Danio rerio*)

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Dissertação apresentada ao Programa de Pós-graduação em Neurociências do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para obtenção do título de Mestre em Neurociências.

Orientador: Prof. Dr. Angelo Piato

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Hibike! Euphonium

S01E12 - 16:10 - 16:52

K-ON!

S02E01 - 00:17 - 01:38

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 about the effects of exposure to OTA on the central nervous system are still scarce. Zebrafish (*Danio rerio*) is a teleost often used in translational research due to its physiological, genetic, and behavioral homology with mammals, in addition to being useful as an environmental bioindicator. 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) 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 stress. 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) activities and decreased non-protein thiols (NPSH) levels in the zebrafish brain. This study showed that OTA can affect brain function in zebrafish even at low doses.

RESUMO

A ocratoxina A (OTA) é uma micotoxina produzida por espécies de fungos filamentosos amplamente encontrada como contaminante em alimentos e com alto potencial tóxico. Estudos demonstraram que essa toxina causa danos aos rins e ao fígado; no entanto, os dados sobre os efeitos da exposição à OTA no sistema nervoso central ainda são escassos. O peixe-zebra (*Danio rerio*) é um teleósteo muito utilizado em pesquisas translacionais devido à sua homologia fisiológica, genética e comportamental com os mamíferos, além de ser útil como bioindicador ambiental. Assim, este estudo teve como objetivo investigar os efeitos da exposição à OTA nos parâmetros comportamentais e neuroquímicos em peixes-zebra adultos. Os animais foram tratados com diferentes doses de OTA (1,38, 2,77 e 5,53 mg/kg) e submetidos a avaliações comportamentais no teste de tanque aberto e teste de interação social. Posteriormente eles foram eutanasiados e os encéfalos usados para avaliar marcadores neuroquímicos associados ao estresse oxidativo. No teste de tanque aberto, OTA alterou a distância percorrida, o ângulo de giro absoluto, a velocidade média e o tempo de congelamento. No entanto, não foram observados efeitos significativos no teste de interação social. Além disso, OTA aumentou as atividades da glutationa peroxidase (GPx), glutationa-S-transferase (GST) e glutationa redutase (GR), também diminuiu os níveis de tióis não-proteicos (NPSH) no encéfalo do peixe-zebra. Este estudo mostrou que a OTA pode afetar o sistema nervoso central em peixes-zebra, mesmo em doses baixas.

LISTA DE ABREVIATURAS

μg - Micrograma

CAT - Catalase

GPx - Glutationa peroxidase

GR - Glutationa redutase

GSH - Glutationa

GST - Glutationa -S-transferase

kg - Quilograma

LD50 - Dose letal mediana

mg - Miligrama

ng - Nanograma

NPSH – Tióis não-proteicos

OECD - Organização para a Cooperação e Desenvolvimento Econômico

OTA - Ocratoxina A

ROS – Espécies reativas de oxigênio

SOD – Superóxido dismutase

TBARS - Substâncias reativas ao ácido tiobarbitúrico

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1. INTRODUÇÃO

1.1 Micotoxinas

Estima-se que cerca de 200 mil pessoas sejam adicionadas diariamente à demanda mundial por alimentos (UNITED NATIONS ENVIRONMENT PROGRAMME; GRID--ARENDAL, 2009). Isso, somado às projeções de que até 2050 o mundo alcance 9,8 bilhões de habitantes (UNITED NATIONS, 2017), torna cada vez mais emergencial a busca por aperfeiçoamentos na produção de alimentos para suprir tais necessidades.

Entretanto, as metodologias que deveriam ser uma solução, têm se tornado um problema ainda maior. O advento do uso de agrotóxicos em grandes plantações já causa sérios impactos ambientais e de saúde pública (LANGLEY; MORT, 2012; WORLD HEALTH ORGANIZATION, 2006). A adulteração fraudulenta de produtos pecuários (CAVIN et al., 2018; XIN; STONE, 2008) coloca em risco a integridade dos consumidores. O investimento da indústria em alimentos processados tem sido relacionado com o aumento na incidência de obesidade, diabetes, doença celíaca e doenças cardíacas (AGUAYO-PATRÓN; CALDERÓN DE LA BARCA, 2017; ANAND et al., 2015; CANELLA et al., 2014); Além disso, as condições ambientais e de manejo inadequado dos produtos alimentícios têm propiciado a presença cada vez maior de micotoxinas (MARROQUÍN-CARDONA et al., 2014).

As micotoxinas são compostos de ocorrência natural provenientes de espécies de fungos e com grande potencial tóxico (TOLA; KEBEDE, 2016). Elas são consideradas metabólitos secundários de diversas espécies, principalmente dos *gêneros Aspergillus, Penicillium e Fusarium*, afetando variados tipos de alimentos e causando os mais diversos efeitos tóxicos, conforme sumarizado na Tabela 1.

Tabela 1: Principais toxinas com seus respectivos fungos, produtos e efeitos relacionados.

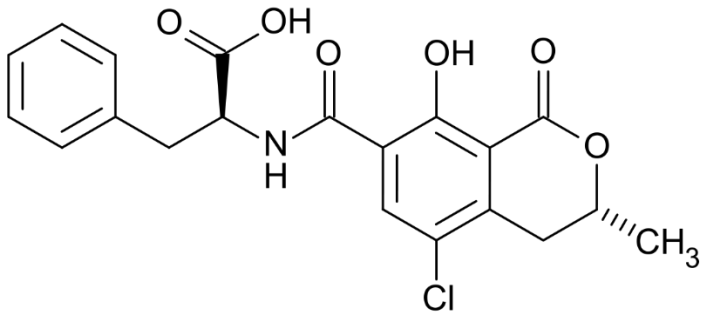
Principais produtos	**Principais fungos**	**Principal toxina**	**Efeitos relacionados (organismo)**
Amendoim e milho	*Aspergillus flavus* *Aspergillus parasiticus*	Aflatoxina B1	Hepatotóxica, nefrotóxica carcinogênica (humanos)
Trigo, aveia, cevada, milho e arroz	*Penicillium citrinum*	Citrina	Nefrotoxicidade (suínos)
Centeio e grãos em geral	*Claviceps purpúrea*	Ergotamina	Gangrena de extremidades e convulsões (humanos)
Milho	*Fusarium verticilliodes*	Fumonisinas	Câncer de esôfago (humanos)
Cevada, café e vinho	*Aspergillus ochraceus* *Aspergillus carbonarius*	Ocratoxina	Hepatotóxica, nefrotóxica e carcinogênica (humanos)
Frutas e sucos de frutas	*Penicullium expansum* *Penicillium griseofulvum*	Patulina	Toxicidade vagamente estabelecida (humanos)
Milho, cevada, trigo, aveia e centeio	Fusarium *sp* Myothecium *sp* Strachybotrys *sp* Trischothecium *sp*	Tricotecenos, T2, neosolaniol, fusanona x, nivalenol, deoxivalenol	Hemorragias, vômitos e dermatites (humanos)
Cereais	*Fusarium graminearum*	Zearalenona	Baixa toxicidade; síndrome da masculinização e feminização (suínos)

Fonte: (FOOD INGREDIENTS BRASIL, 2009)

1.2 Ocratoxina A

A ocratoxina A (OTA) é uma micotoxina produzida por espécies de fungos filamentosos e pertencente ao subgrupo das ocratoxinas, juntamente com a ocratoxina B e C, ambas com menor ocorrência natural e baixa toxicidade. OTA, (2S)-2-[[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydroisochromene-7-carbonyl]amino]-3-phenylpropanoic acid, é um composto que apresenta uma ligação amida entre L-fenilalanina e uma diidroisocumarina (Figura 1). Na tabela 2 são elencadas algumas informações físico-químicas sobre a OTA.

Figura 1: Estrutura da ocratoxina A (OTA)



Fonte: PUBCHEM

Tabela 2: Informações físico-químicas da ocratoxina A (OTA)

Nome	Ocratoxina A
CAS	303-47-9
IUPAC	(2*S*)-2-[[(3*R*)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydroisochromene-7-carbonyl]amino]-3-phenylpropanoic acid
Fórmula Molecular	$C_{20}H_{18}ClNO_6$
Peso Molecular	403,815 g/mol
Ponto de fusão	160 ºC

Fonte: PUBCHEM

O primeiro registro dessa substância foi feito em 1965, tendo sido detectada no fungo *Aspergillus ochraceus* (VAN DER MERWE, K.; STEYN, P.; FOURIE, L.; SCOTT, D.; THERON, 1965) e mais tarde em outras espécies de *Aspergillus* (*A. niger, A. tubingensis, A.*

westerdijkiae, A. steynii, A. lacticoffeatus, A. sclerotionige e *A. carbonarius*) e também no gênero *Penicillium* (*P. verrucosum* e *P. nordicum*) (MALIR et al., 2016).

Os mecanismos envolvidos na síntese de OTA ainda não foram elucidados. A síntese de OTA tende a ocorrer em situações condicionadas que envolvem a ocorrência de umidade e temperatura entre 15°C e 30°C (AMÉZQUETA et al., 2012). Durante o processo acredita-se que a enzima policetídeo sintase seja responsável pela reação do acetato com malonato, levando a formação de diidrosocumarina. A maleína, precursor da OTA, é oxidada à 7-carvoxi-meleína, também chamada de ocratoxina β, a qual será transformada em ocratoxina α por uma cloração mediada por cloroperoxidase. A fenilalanina, outro precursor, é esterificado e juntamente ao malonato já ativado irá gerar ocratoxina C. Por fim, a desesterificação de ocratoxina C formará OTA. Da mesma forma existem muitas lacunas com relação ao mecanismo de toxicidade desta toxina. A hipótese mais bem difundida envolve a inibição da síntese proteica devido à competição entre o grupo fenilalanina presente na OTA e o aminoácido fenilalanina, essencial para o funcionamento deste sistema (KŐSZEGI; POÓR, 2016).

Devido à sua ocorrência natural, especialmente em determinadas condições climáticas e do armazenamento inadequado de insumos, a OTA se tornou um contaminante bastante comum em alimentos e no ecossistema. Há evidencias da presença de OTA no ambiente, com a micotoxina já tendo sido detectada em fontes de água (HU et al., 2017; MATA et al., 2015) e em animais marinhos (SUN et al., 2015). A maior incidência de detecção, no entanto, ocorre nos alimentos. OTA já foi encontrada em diversos tipos de alimentos no mundo, incluindo carnes em mercados na Croácia (PLEADIN et al., 2015), no café brasileiro e europeu (ALMEIDA et al., 2007; V. D. STEGEN et al., 1997), vinhos e cervejas do Chile e Hungria (VARGA et al., 2014; VEGA et al., 2012), frutas na Argentina e Canadá (LOMBAERT et al., 2004; MAGNOLI et al., 2004) e sucos europeus (JØRGENSEN, 2005).

Dentre os alimentos mais afetados pela presença da toxina estão os cereais (CZERWIECKI; CZAJKOWSKA; WITKOWSKA-GWIAZDOWSKA, 1998; J∅RGENSEN; RASMUSSEN; THORUP, 1992; LEE; RYU, 2015; VILLA; MARKAKI, 2009). Curiosamente, dados indicam que a presença de OTA é maior em cereais produzidos de forma orgânica (JØRGENSEN; JACOBSEN, 2002), método esse que visa oferecer maior segurança alimentar aos consumidores, mas que possivelmente favorece o crescimento dos organismos produtores da toxina devido ao baixo uso de fungicidas e outros agrotóxicos. A possível toxicidade atribuída especificamente a essa classe de alimentos se torna ainda mais preocupante quando se leva em conta o fato de que cereais contribuem com boa parte da alimentação de

crianças ao longo do desenvolvimento. OTA já foi detectada em preparações alimentícias para bebês (BONERBA et al., 2017), mas ainda não está elucidado os possíveis efeitos que a exposição precoce à toxina possa acarretar no desenvolvimento da criança e possíveis consequências para a vida adulta.

OTA é a toxina do grupo das ocratoxinas mais prevalente e tóxica (BRIEN; DIETRICH, 2004). O efeito dessa toxina já foi avaliado em roedores (CASTEGNARO et al., 1998; KANISAWA; SUZUKI, 1978), aves (STOEV, 2010) e peixes (DOSTER; SINNHUBER; PAWLOWSKI, 1974; MANNING et al., 2003). Sua presença tem sido associada com imunossupressão (LEA; STEIEN; STORMER, 1989), hepatotoxicidade (QI et al., 2015), nefrotoxicidade e nefropatologias, especialmente a Nefropatia Endémica dos Balcãs (ABID et al., 2003; FUCHS; PERAICA, 2005). Além disso, OTA também tem sido cada vez mais associada a quadros neuropsiquiátricos como a encefalomielite miálgica, neurotoxicidade e sintomatologia parkinsoniana (BREWER et al., 2013; SAVA et al., 2006b; YOON et al., 2009).

Devido ao potencial tóxico da OTA, os países têm feito esforços para regulamentar e tentar controlar a presença dessa toxina em produtos alimentícios (BRASIL; SAÚDE; ANVISA, 2011; CANADA BUREAU OF CHEMICAL SAFETY, FOOD DIRECTORATE, 2009; EUROPEAN UNION, 2014). A Organização Mundial da Saúde (OMS) revisou em 2001 as questões pertinentes aos limites aceitos para a presença de OTA em alimentos, determinando que a ingestão semanal tolerável (*Provisional tolerable weekly intake* - PTWI) seria de 100 ng/kg, entretanto, esse valor varia de acordo com as normas internas definidas pelos países e com o porte físico do indivíduo (WORLD HEALTH ORGANIZATION, 2002). Esse método gera divergências nos valores "aceitos" como limites, assim, prejudicando a fiscalização e gerando lacunas nas normas dos órgãos reguladores. Essa falta de padronização quanto aos valores aceitáveis se torna ainda mais grave devido à natureza exponencial da produção alimentícia. Em 2019, a produção agrícola foi responsável por movimentar bilhões de toneladas em alimentos produzidos (FOOD AND AGRICULTURE ORGANIZATION (FAO), 2019a) e um enorme montante em dólares negociados através de exportações (FOOD AND AGRICULTURE ORGANIZATION (FAO), 2019b). Nesse cenário países como China, Estados Unidos, Índia, Alemanha e Japão são os maiores responsáveis pelas importações mundiais de *commodities* alimentícios (FOOD AND AGRICULTURE ORGANIZATION (FAO), 2019c), enquanto os maiores exportadores são Brasil, Estados Unidos, Indonésia, Holanda e França (FOOD AND AGRICULTURE ORGANIZATION (FAO), 2019d). Essas nações líderes no mercado de alimentos são justamente algumas das citadas anteriormente com

casos detectados de OTA em diversos produtos, tornando ainda mais importante o controle dos níveis dessas toxinas nos alimentos que chegam ao consumidor, afinal, essas substâncias desconhecem fronteiras e transitam entre os continentes.

1.3 Peixe-zebra como organismo modelo

O peixe-zebra (*Danio* rerio, Hamilton-Buchanan, 1822), conhecido mundialmente como *zebrafish*, vem sendo amplamente utilizado como organismo modelo em pesquisas nas áreas de farmacologia (TRAN et al., 2017), toxicologia (GERLAI; LEE; BLASER, 2006) e neurociências (BENVENUTTI et al., 2018; PANCOTTO et al., 2018).

Nativo do sul asiático, o peixe-zebra é um teleósteo que tem ampla homologia genética e fisiológica com os humanos (LIESCHKE; CURRIE, 2007). O peixe-zebra também é um organismo no qual se pode facilmente mimetizar as condições de um animal em seu ecossistema natural. Além disso, o peixe-zebra possui embriões transparentes que possibilitam a observação do desenvolvimento embrionário até estágios larvais (STEWART et al., 2014). Por todos esses fatores, o peixe-zebra tem ganhado espaço em estudos ecotoxicológicos (HERMSEN et al., 2012; ROY; CARNEIRO; OCHS, 2016; VALADAS et al., 2019; WIEGAND et al., 2001). Porém, existem poucos dados sobre os efeitos comportamentais e bioquímicos da exposição à Ocratoxina A em peixe-zebra adulto.

2. OBJETIVOS

2.1 Objetivos gerais

Avaliar os efeitos da exposição à OTA sobre parâmetros comportamentais e bioquímicos em peixes-zebra adultos.

2.2 Objetivos específicos

Avaliar os efeitos da exposição à OTA sobre:

a) Parâmetros comportamentais no teste de tanque aberto em peixes-zebra adultos;
b) Parâmetros comportamentais no teste de interação social em peixes-zebra adultos;
c) Parâmetros bioquímicos relacionados ao estresse oxidativo como peroxidação lipídica (TBARS), tióis não-proteicos (NPSH), glutationa peroxidase (GPx), glutationa redutase (GR) e glutationa S-transferase (GST).

3. ARTIGO CIENTÍFICO

O artigo a seguir intitulado *Ochratoxin A induces locomotor impairment and oxidative imbalance in adult zebrafish* foi submetido a um periódico.

Ochratoxin A induces locomotor impairment and oxidative imbalance in adult zebrafish

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3.1 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. Zebrafish (*Danio rerio*) is a teleost often used in translational research due to its physiological, genetic, and behavioral homology with mammals, in addition to being useful as an environmental bioindicator. 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) 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) activities and decreased non-protein thiols (NPSH) levels in the zebrafish brain. This study showed that OTA can affect brain function in zebrafish even at low doses.

3.2 Introduction

It is estimated that 200 thousand people are added daily to the world's demand for food (NELLEMANN; UNITED NATIONS ENVIRONMENT PROGRAMME; GRID--ARENDAL, 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 of pesticide use in large crops is already causing serious environmental and public health impacts (LANGLEY; MORT, 2012; RANI et al., 2021; WORLD HEALTH ORGANIZATION, 2006). Improperly tampering with livestock products may put consumers' lives in danger (CAVIN et al., 2018; XIN; STONE, 2008), and industry investment in processed foods has been linked to the incidence of obesity, diabetes, celiac disease, and heart disease (AGUAYO-PATRÓN; CALDERÓN DE LA BARCA, 2017; ANAND et al., 2015; CANELLA et al., 2014). 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; KEBEDE, 2016). Ochratoxin A (OTA) is a mycotoxin produced by filamentous fungi and belongs to the ochratoxin subgroup, along with ochratoxin 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 (HU et al., 2017; MATA et al., 2015) 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), in Brazilian and European coffee (ALMEIDA et al., 2007; V. D. STEGEN et al., 1997), wines, and beers from Chile and Hungary (VARGA et al., 2014; VEGA et al., 2012), fruits in Argentina and Canada (LOMBAERT et al., 2004; MAGNOLI et al., 2004), European juices (JØRGENSEN, 2005) and several other types of products across the globe.

The exportation market moves around billions of dollars per year (FOOD AND AGRICULTURE ORGANIZATION (FAO), 2019b) and billions of food tons (FOOD AND AGRICULTURE ORGANIZATION (FAO), 2019a) are transported to countries with varying laws and cultures. Most of the nations have protocols and specific regulations for the tolerable

limits of contaminants in the food, including OTA (BUREAU OF CHEMICAL SAFETY; FOOD DIRECTORATE; CANADA HEALTH PRODUCTS AND FOOD BRANCH, 2009; MINISTÉRIO DA SAÚDE; AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, 2011; OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2006). 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 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; POÓR, 2016). The effects of OTA have already been evaluated in rodents (CASTEGNARO et al., 1998; KANISAWA; SUZUKI, 1978), birds (STOEV, 2010), and fish (DOSTER; SINNHUBER; PAWLOWSKI, 1974; MANNING et al., 2003). The toxin has been associated with immune modulation (LEA; STEIEN; STORMER, 1989), hepatic (Qi et al., 2015), and kidney diseases (ABID et al., 2003; FUCHS; PERAICA, 2005). OTA has also been increasingly associated with neuropsychiatric disorders (BREWER et al., 2013; SAVA et al., 2006b; YOON et al., 2009). 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; CURRIE, 2007). For this reason, this species has been used as a research animal model for different fields such as embryology and development (HAO et al., 2013; KELLER et al., 2008), oxidative stress (CHOI et al., 2010; MARCON et al., 2018), behavior (ABOZAID et al., 2020; NABINGER et al., 2021; REIS et al., 2020) and genetics (Nasevicius and Ekker, 2000; 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; PARK et al., 2020; VALADAS et al., 2019). 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.

3.3 Materials and methods

3.3.1 Animals

The experiments were performed using 96 adult short-fin wild-type zebrafish (*Danio rerio*, Hamilton, 1822) of both sexes (50:50 male:female ratio) obtained from the local commercial supplier. 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).

3.3.2 Drugs

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; SINNHUBER; WALES, 1972) since there are no similar studies on adult zebrafish. The established doses for OTA in this study were 1.38, 2.77, and 5.53 mg/kg.

3.3.3 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 hours) 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) x Epidurakatheter 0.45 × 0.85 mm (Perifix®- Katheter, Braun, Germany) x 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 seconds (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 hours. After 96 hours 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 hours. 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 parameters analyses were: 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.

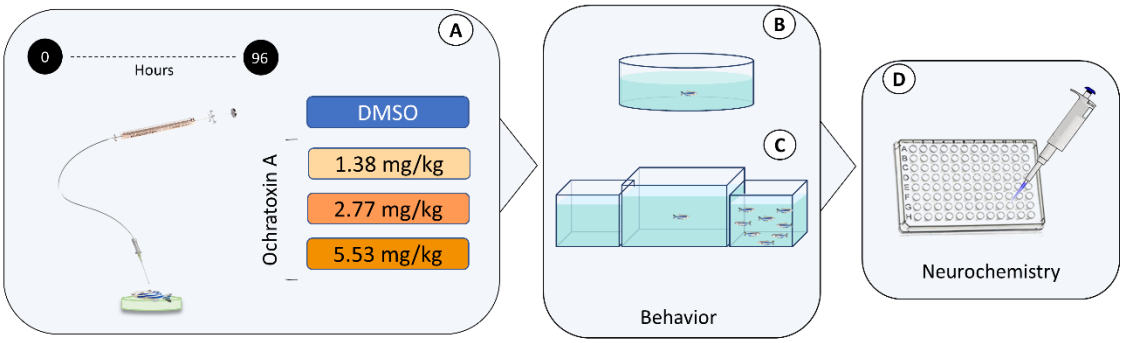


Figure 1. Experimental design.

3.3.4 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.

3.3.5 Social interaction test (SIT)

In the SIT, fish were placed individually in a central tank (30 x 10 x 15 cm) flanked by two identical tanks (15 x 10 x 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 analyzes 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.

3.3.6 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 sample, a pool of 4 brains was used (n = 6) and mixed with 600 µL of phosphate-buffered saline (PBS, pH 7.4, Sigma-Aldrich). The homogenate was centrifuged at 2400 g for 10 min at 4 °C and the supernatants were collected for the analyses of the following

parameters: lipid peroxidation (TBARS) (Sachett, 2020), non-protein thiols (NPSH) (SACHETT, 2020), and glutathione peroxidade (GPx) (SACHETT, 2021a), glutathione reductase (GR) (SACHETT, 2021b) and glutathione-S-transferase (GST) (HABIG; JAKOBY, 1981).

3.3.7 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 data sets using D'Agostino-Pearson and Levene tests, respectively. The student's t-test was performed to compare 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 $p<0.05$.

3.4 Results

DMSO did not show important modulation on behavior (Supplementary material 1) or induce oxidative damage (Supplementary material 2) compared with sodium chloride control. Therefore, we only used DMSO as a control group.

3.4.1 Open tank test

Fig. 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 damage. The parameters of crossings and freezing episodes were not altered by any dose.

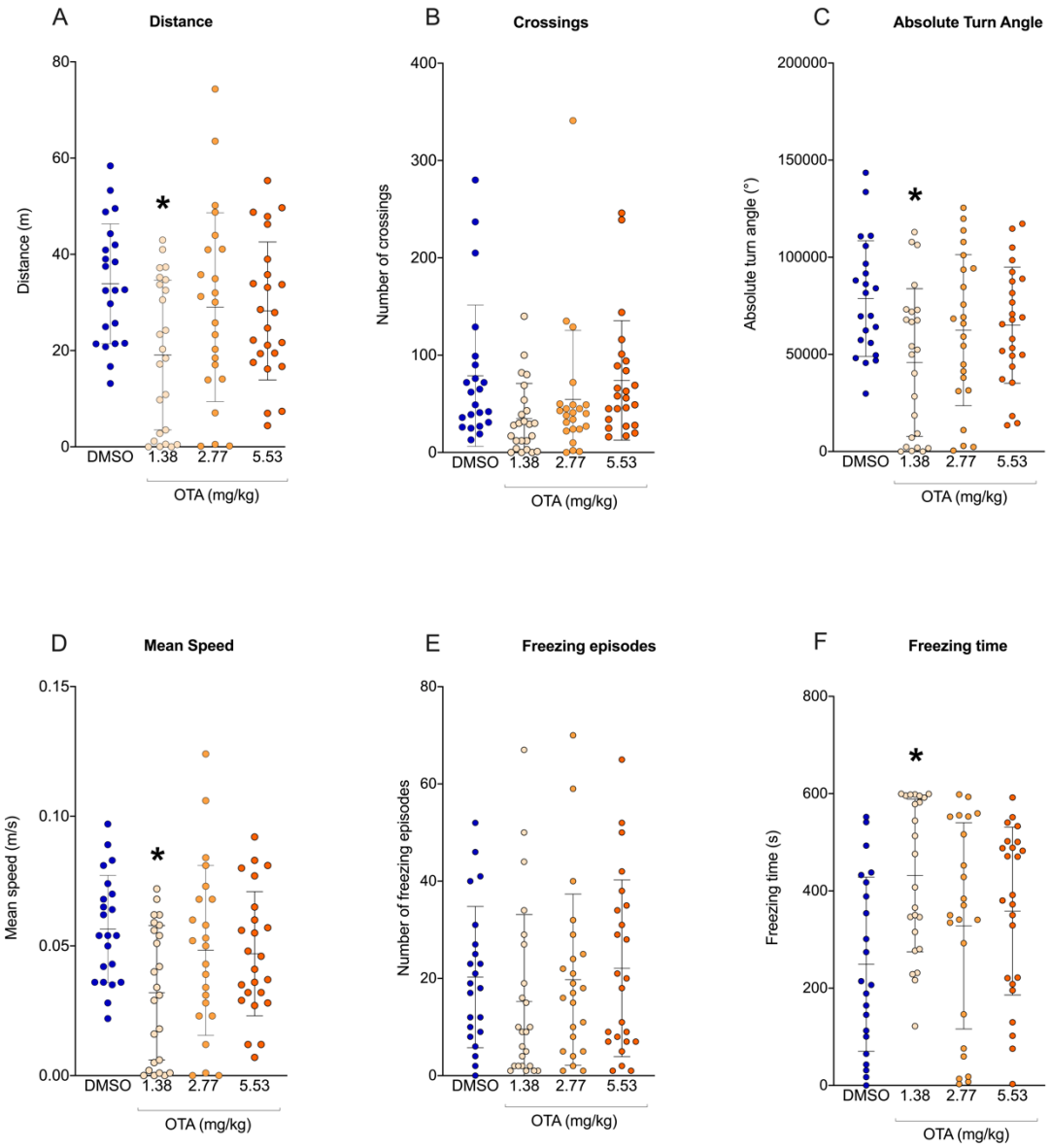


Figure 2. Effects of OTA in the open tank test. (A) distance, (B) crossings, (C) absolute turn angle, (D) mean speed, (E) freezing episodes, and (F) freezing time. Data are expressed as mean ± standard deviation (S.D.). n=22-24. One-way ANOVA followed by Tukey's post hoc test. *p < 0.05.

3.4.2 Social interaction test

Fig. 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.

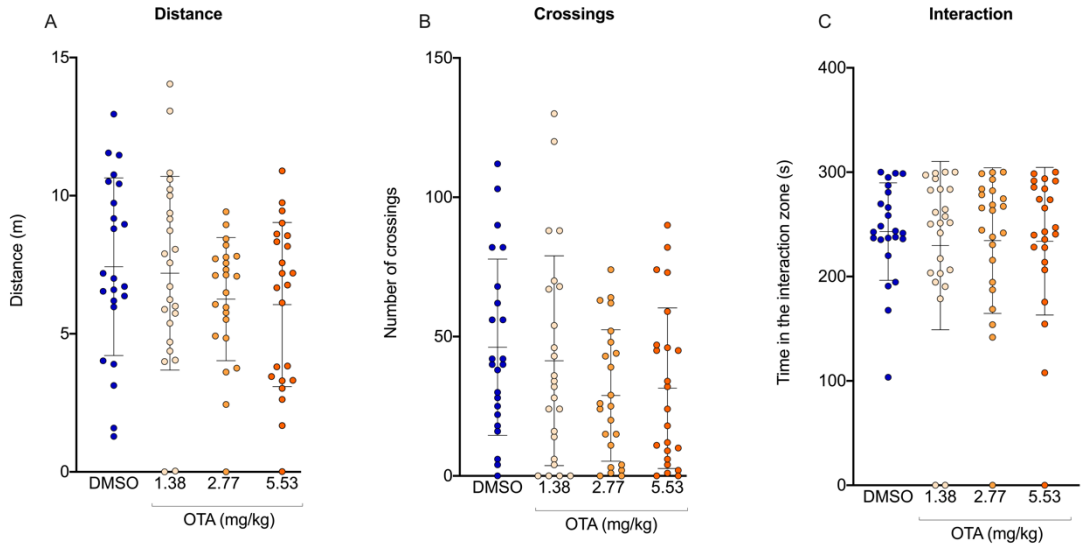


Figure 3. Effects of OTA in the social interaction test. (A) distance, (B) crossings, (C) interaction time. Data are expressed as mean ± standard deviation (S.D.). n=23-24. One-way ANOVA.

3.4.3 Neurochemical analysis

Fig. 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, $p = 0.0397$) activities. 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. 4D, $p = 0.0146$) activities. The dose of 5.53 mg/kg increased GPx (Fig. 4C, $p < 0.0001$) and GR (Fig. 4E, $p = 0.0238$) activities.

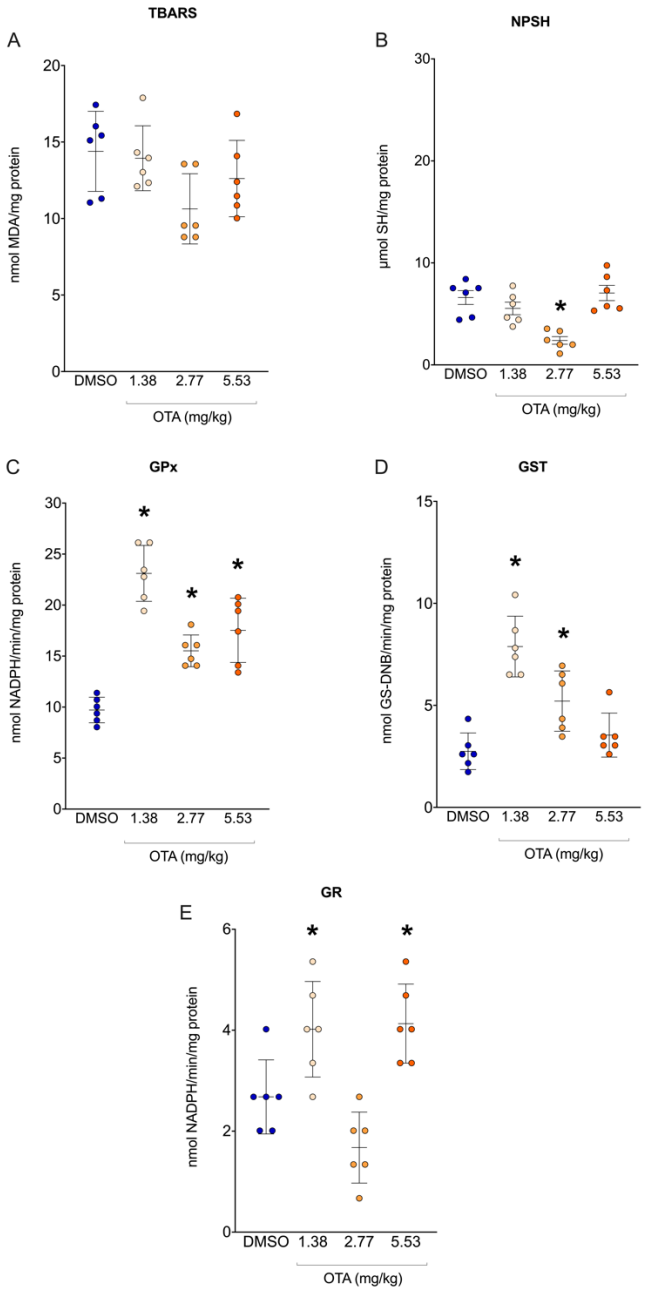


Figure 4. Effects of OTA in neurochemical parameters. (A) TBARS, (B) NPSH, (C) GPx, (D) GST, and (E) GR. Data are expressed as mean ± standard deviation (S.D.). n= 6. One-way ANOVA followed by Tukey's post hoc test. *$p < 0.05$.

3.5 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.

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 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, 2006b). 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; GERLAI, 2011; DREOSTI et al., 2015; SCERBINA; CHATTERJEE; GERLAI, 2012). 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; BRACARENSE; OSWALD, 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

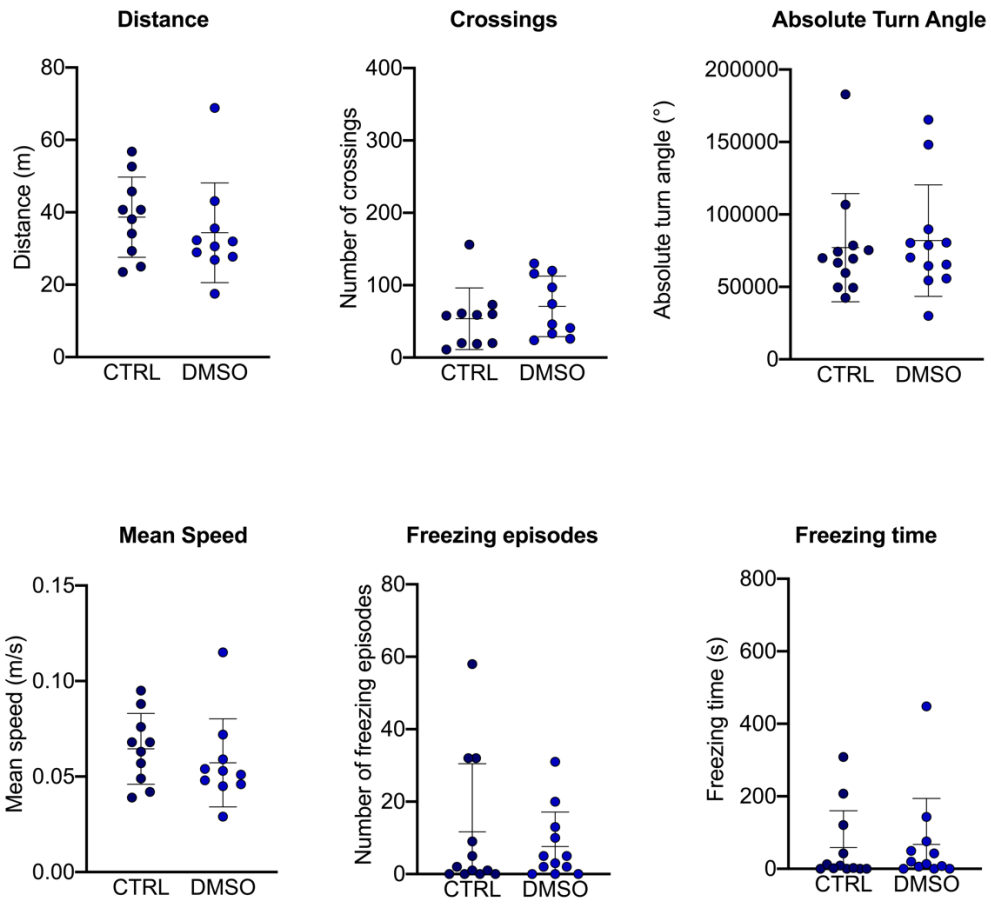
hydrogen peroxide (H_2O_2). H_2O_2 by Fenton reaction produces a hydroxyl radical ($OH^{\bullet}$) that is responsible for oxidative damage (ADLOUNI et al., 2000). Another common pathway for OTA is the formation of an OTA–Fe^{3+} complex that is reduced in OTA- Fe^{2+} by cytochrome P450 resulting in $OH^{\bullet}$ (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; SIEBENMANN; PIETSCH, 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 activity 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 (FERNYE et al., 2021; KÖVESI et al., 2019). 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 of GST activity 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) (DASURI; ZHANG; KELLER, 2013; GANDHI; ABRAMOV, 2012).

Despite the zebrafish being a model used for decades in research in several areas, many gaps still exist about 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

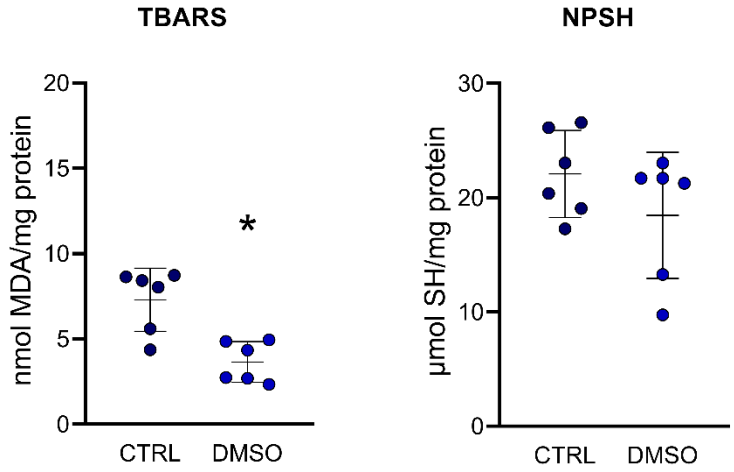
analysis in fish (Gonçalves et al., 2020), including the OECD protocols (OECD Guidelines for the Testing of Chemicals, 1992). However, for adult animals, the methodologies tend to be limited to direct exposure to the animals' water, which is not suitable for all protocols. In the case of OTA, the formulation 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) and neurotoxicity in the development of mice (Miki et al., 1994; Tamaru et al., 1988). In fish, OTA was injected peritoneally into rainbow trout (*Salmo gairdneri*) acutely (96 hours) 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.

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 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; 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; PAPADIMITRIOU; KIZIL, 2015; GHOSH; 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.

3.6 Supplementary material



Supplementary material 1. Comparison between CRTL (sodium chloride) vs. DMSO (dimethyl sulfoxide) on behavior parameters at open tank test. Data are expressed as mean ± standard deviation (S.D.). n=10. Student's t-test. *p < 0.05.



Supplementary material 2. Comparison between CRTL (sodium chloride) vs. DMSO (dimethyl sulfoxide) on neurochemical parameters. Data are expressed as mean ± standard deviation (S.D.). n=6. Student's t-test. *$p < 0.05$.

3.7 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 change and neurochemical modulation, 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.

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4. DISCUSSÃO GERAL

Esse estudo demonstrou os efeitos deletérios da Ocratoxina A (OTA) em peixe-zebra adulto. OTA afetou o comportamento dos animais no teste de tanque aberto, diminuindo a distância total percorrida, velocidade média, ângulo absoluto de virada e causou aumento no tempo de congelamento. Entretanto, no teste de interação social não houve alterações comportamentais. As análises bioquímicas mostraram que a toxina é capaz de gerar alterações nos parâmetros de estresse oxidativos que embora não tenha sido o suficiente para alterar TBARS, foi capaz de acionar as defesas oxidativas.

Existem escassos dados na literatura sobre os efeitos comportamentais relacionados à exposição à OTA não apenas em peixes, mas também em outros modelos animais. Em larvas de peixe-zebra, a OTA diminuiu a velocidade de nado dos animais, mas não alterou parâmetros de distância e tempo de atividade (KHEZRI et al., 2018). Em roedores foi mostrado que a OTA injetada por via intraperitoneal foi capaz de causar alterações comportamentais em análise de marcha, atividade espontânea, teste de cilindro e pole teste, semelhante a aos sintomas presentes em pacientes com doença de Parkinson que foi estabilizada com o uso de L-DOPA (BHAT et al., 2018). Aqui foi mostrado a interferência de OTA nos parâmetros de locomoção em peixe-zebra no teste de tanque aberto, semelhante aos resultados anteriormente citados em outros modelos. Uma possiblidade para estes achados poderia ser a relação entre a locomoção e a via nigro-estriatal que já foi relatada ser afetada por OTA em roedores (SAVA et al., 2006a, 2006b). Entretanto, não houve alterações nos parâmetros de interação social. O comportamento social em peixe-zebra apresenta uma coesão de cardume que visa a busca por alimento, a fuga de predadores e a reprodução da espécie (PITCHER, 1993). Desta forma, por ser um modelo muito atrelado às funções sociais, o peixe-zebra tem sido muito estudado com relação a este tipo de comportamento (BUSKE; GERLAI, 2011; DREOSTI et al., 2015; SCERBINA; CHATTERJEE; GERLAI, 2012). Entretanto, justamente pela socialização ser geneticamente preservada e de caráter ontogênico nessa espécie, pode ser um parâmetro menos vulnerável a modulações mais brandas como as demonstradas neste estudo, uma vez que a concentração mais baixa utilizada equivaleu a 25% da DL50 estabelecida em outro modelo. Outro aspecto a ser considerado é com relação às pistas fornecidas pelo aparato, uma vez que estudos anteriores já demonstraram o caráter multifatorial do comportamento social no peixe-zebra, sendo atrelado a pistas visuais (ENGESZER et al., 2007), olfatórias (GERLACH et al., 2007) e também sensível a substâncias de alarme liberadas pelos coespecíficos (CANZIAN et al.,

2017). O aparato utilizado neste estudo, entretanto, permitia apenas que as pistas visuais fossem transmitidas ao animal, assim, é incerto afirmar quais seriam os efeitos da OTA sob outros parâmetros envolvidos no comportamento social do animal.

Com o aumento da preocupação global com a disseminação das micotoxinas, o efeito destes compostos sobre os parâmetros de estresse oxidativo se tornou um assunto muito debatido (DA SILVA; BRACARENSE; OSWALD, 2018; MAVROMMATIS et al., 2021), com destaque para a Ocratoxina A (SORRENTI et al., 2013; TAO et al., 2018). OTA pode interagir com peroxidases que produzem radical fenoxil. A glutationa (GSH) é capaz de transformar o radical fenoxil em OTA novamente, formando um radical ânion superóxido ($O_2^{\bullet-}$) que resulta em peróxido de hidrogênio (H_2O_2). H_2O_2, pela reação de Fenton, produz um radical hidroxila ($OH^{\bullet}$) que é responsável pelo dano oxidativo (ADLOUNI et al., 2000). Outro caminho comum para OTA é a formação de um complexo OTA-Fe^{3+} que é reduzido em OTA-Fe^{2+} pelo citocromo P450 resultando em $OH^{\bullet}$ (RAHIMTULA et al., 1988). Em larvas de peixe-zebra houve a formação de espécies reativas de oxigênio proporcional ao aumento da concentração de OTA (TSCHIRREN; SIEBENMANN; PIETSCH, 2018). Um estudo com tambaqui (*Colossoma macropomum*), um peixe de água doce, verificou o aumento da formação de espécies reativas de oxigênio e da peroxidação lipídica nos músculos do animal, assim como diminuição na atividade de SOD (superóxido dismutase) e GPx (BALDISSERA et al., 2020). De forma semelhante, foi visto o aumento da peroxidação lipídica, CAT (catalase) e GR com uma diminuição na atividade da enzima SOD e GSH no cérebro, rim e fígado de ratos. (NOGAIM et al., 2020). Foi verificado o aumento na formação de espécies reativas de oxigênio, peroxidação lipídica e diminuição de GSH em células de rim (LEE et al., 2018). No entanto, estudos em aves mostraram que em exposições de longo prazo as defesas antioxidantes podem aumentar frente ao desbalanço oxidativo, especialmente o sistema da glutationa (FERNYE et al., 2021; KÖVESI et al., 2019). Neste estudo nós mostramos que em peixe-zebra adulto houve um aumento das defesas enzimáticas com elevação de GPx, GR e GST, especialmente na dose mais baixa. Na dose intermediaria não houve aumento em GR como ocorrido nas outras doses, o que condiz com a diminuição dos níveis de NPSH neste grupo uma vez que a GR é a responsável pela reciclagem da glutationa que é essencial para a manutenção dos níveis de NPSH. O aumento da GPx nestas condições indica uma tentativa de controlar um possível aumento das espécies reativas de oxigênio, algo bastante comum de ocorrer em exposições à OTA conforme mencionados nos estudos anteriores. Da mesma forma, essa ativação das defesas preveniu a peroxidação lipídica por TBARS e a diminuição de GST.

Apesar do peixe-zebra ser usado há décadas na pesquisa em diversas áreas, muitas lacunas ainda existem com relação ao modelo, especialmente na área de toxicologia. Nos últimos anos houve um aumento considerável de estudos nesse campo devido a iniciativas de padronizar esse tipo de análise em peixes (Gonçalves et al., 2020), incluindo os protocolos da OECD (OECD Guidelines for the Testing of Chemicals, 1992). Entretanto, para animais adultos as metodologias sugeridas são limitadas à exposição direta na água dos animais, o que não é adequado a todas as realidades. No caso da OTA, a formulação do composto e a dificuldade em armazenar ou descartar os resíduos tornaram inviáveis esse tipo de protocolo de exposição, assim foi optado nesse trabalho pela injeção intraperitoneal que é padronizada no laboratório. O uso da injeção intraperitoneal para avaliar os efeitos da OTA já foi empregado em outros modelos, sendo eficaz para detectar efeitos deletérios ao mecanismo de metabolização em ratos (Størmer et al., 1985) e neurotoxicidade no desenvolvimento de camundongos (Miki et al., 1994; Tamaru et al., 1988). Em peixes, OTA foi injetada por via intraperitoneal na truta arco-íris (*Salmo gairdneri*) de forma aguda (96h) para avaliação toxicológica por histologia e determinação da DL50 (5,53 mg/kg) (Doster et al., 1974). No entanto, esses dados nunca foram aprofundados em outras espécies e o uso do peixe-zebra para avaliar os efeitos da OTA se manteve limitado, havendo poucas informações quantos aos efeitos da toxina em animais adultos e menos ainda para modulações neurotóxicas.

Devido a essas lacunas importantes na literatura, outro ponto a ser esclarecido é a reação dose-resposta do peixe-zebra frente à OTA. Neste estudo as doses que se mostraram mais reativas a nível comportamental e bioquímico foram as mais baixas, tendo a dose mais alta alterado poucos parâmetros bioquímicos. Assim, neste estudo a OTA mostrou uma característica hormética em peixe-zebra adulto. Hormese é um comportamento bifásico de dose-resposta caracterizado pelo estimulo frente a doses baixas e inibição com doses altas (CALABRESE; BALDWIN, 2002; MATTSON, 2008). Em OTA esse tipo de curva já foi relatado em estudo *in vitro* (LI et al., 2014), porém, esta foi a primeira vez que este comportamento foi visto em um modelo *in vivo*. Uma curva bifásica pode indicar a plasticidade biológica do organismo alvo (CALABRESE; MATTSON, 2011) e sendo o peixe-zebra um modelo muito estudado justamente por sua capacidade de neuroplasticidade e regeneração (COSACAK; PAPADIMITRIOU; KIZIL, 2015; GHOSH; HUI, 2016), é possível que o comportamento hormético da OTA neste caso esteja ligado às características biológicas do animal. Além disso, curvas horméticas costumam ocorrer com disruptores endócrinos (VANDENBERG et al., 2012) e outros estudos já demonstraram o potencial da OTA para

interferir na produção hormonal (FRIZZELL et al., 2013; WOO et al., 2013). Por tudo isso, os resultados toxicológicos para doses baixas não devem ser ignorados.

5. CONCLUSÃO

Apesar do investimento nos esforços para controlar a disseminação das micotoxinas e o aumento de estudos com relação aos efeitos destes compostos, muito ainda é necessário para que o cenário ideal seja alcançado. Este estudo mostrou que a Ocratoxina A pode causar efeitos deletérios no peixe-zebra adulto, afetando seu comportamento e os parâmetros bioquímicos. Entretanto, mais estudos são necessários para esclarecer o exato mecanismo de ação do composto neste modelo animal e assim oferecer uma base ecotoxicológica mais ampla.

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7. ANEXOS

7.1 Carta de aprovação da Comissão de Ética no Uso de Animais da UFRGS

U F R G S
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL

PRÓ-REITORIA DE PESQUISA

Comissão De Ética No Uso De Animais

CEUA UFRGS

CARTA DE APROVAÇÃO

Comissão De Ética No Uso De Animais analisou o projeto:

Número: 37761
Título: Efeitos da exposição à ocratoxina a sobre parâmetros comportamentais e bioquímicos em peixes-zebra (Danio rerio)

Vigência: 01/09/2019 à 01/09/2021

Pesquisadores:

Equipe UFRGS:

ÂNGELO LUIS STAPASSOLI PIATO - coordenador desde 01/09/2019
Ana Paula Herrmann - coordenador desde 01/09/2019
Jessica Valadas da Silva - Aluno de Mestrado desde 01/09/2019

Comissão De Ética No Uso De Animais aprovou o mesmo , em reunião realizada em 06/01/2020 - Plenarinho - Andar Térreo do Prédio da Reitoria - Campus Centro/UFRGS - AV. Paulo Gama/ RS, em seus aspectos éticos e metodológicos, para a utilização de 504 peixes-zebra (ambos os sexos), provenientes do Departamento de Bioquímica da Universidade Federal do Rio Grande do Sul, de acordo com os preceitos das Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08 de novembro de 2008, o Decreto 6899 de 15 de julho de 2009, e as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), que disciplinam a produção, manutenção e/ou utilização de animais do filo Chordata, subfilo Vertebrata (exceto o homem) em atividade de ensino ou pesquisa.

Porto Alegre, Segunda-Feira, 2 de Março de 2020

ALEXANDRE TAVARES DUARTE DE OLIVEIRA
Coordenador da comissão de ética