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SELENITO DE SÓDIO PREVINE NEUROTOXICIDADE INDUZIDA POR PARAQUAT EM PEIXE-ZEBRA (*Danio rerio*)

Santa Maria, RS, Brasil.

2016

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Dissertação apresentada ao Curso de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do título de **Mestre em Bioquímica Toxicológica.**

Orientadora: Profª. Drª. Vania Lucia Loro
Co-orientador: Prof. Dr. Denis Broock Rosemberg

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DEDICATÓRIA

Dedico este trabalho as pessoas que me fizeram ter coragem para enfrentar todos os desafios com garra e determinação: meus pais, minha irmã, minha sobrinha e meu primo!

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(Viagem ao centro da Terra - Julio Verne)

RESUMO

SELENITO DE SÓDIO PREVINE NEUROTOXICIDADE INDUZIDA POR PARAQUAT EM PEIXE-ZEBRA (*Danio rerio*)

AUTOR: Talise Ellwanger Müller

ORIENTADOR: Vania Lucia Loro

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Considerando as propriedades antioxidantes do selenito de sódio (Na_2SeO_3) e o envolvimento dos eventos de estresse oxidativo e nitrosativo na neurotoxicidade induzida pelo herbicida paraquat (PQ), este estudo investigou o potencial efeito protetor de uma dieta suplementada com Na_2SeO_3 sobre parâmetros bioquímicos e comportamentais em peixes-zebra expostos ao PQ. Os peixes foram pré-tratados com uma dieta suplementada com Na_2SeO_3 por 21 dias e após esse período o tratamento com PQ (20 mg/kg) para indução do modelo de neurotoxicidade foi administrado intraperitonealmente, totalizando 6 injeções por 16 dias (uma injeção a cada 3 dias). Entre os parâmetros comportamentais analisados, o pré-tratamento com Na_2SeO_3 atenuou danos locomotores como diminuição da distância percorrida, o aumento do tempo gasto na área do topo do aquário e o aumento na latência para os peixes entrarem na área mais perto dos co-específicos (grupo de interação). Além disso, o pré-tratamento com Na_2SeO_3 aboliu a exacerbação do comportamento de *freezing* e ainda preveniu comportamentos agonísticos do tipo ansiedade e agressividade. Em relação aos danos oxidativos, o pré-tratamento com Na_2SeO_3 preveniu o aumento nos níveis de carbonilação de proteínas (CP), espécies reativas de oxigênio (EROS) e nitrito/nitrato (NOx) causados pelo tratamento com PQ. O pré-tratamento com Na_2SeO_3 também preveniu o aumento nas enzimas antioxidantes catalase (CAT), glutationa peroxidase (GPx) e a diminuição dos níveis de tióis não proteicos (NPSH) induzidos pelo tratamento com PQ. A ativação da enzima glutationa-S-tranferase (GST) tanto pela dieta com Na_2SeO_3 e pelo tratamento com PQ foi eficaz em prevenir alterações nos níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS). Em conclusão, a dieta com Na_2SeO_3 preveniu as alterações comportamentais e danos bioquímicos observados em animais tratados com PQ. O tratamento com Na_2SeO_3 foi benéfico pelo fato de atuar não somente na modulação dos parâmetros redox, mas também na modulação dos fenótipos do tipo ansiedade e agressividade em peixes-zebra tratados com PQ. Este estudo demonstrou pela primeira vez que o peixe-zebra é um organismo modelo conveniente para o *screening* de potenciais moléculas neuroprotetoras contra a neurotoxicidade do PQ.

Palavras-chave: Selênio, Peixe, Herbicida, Comportamento, doença de Parkinson

ABSTRACT

SODIUM SELENITE PREVENTS PARAQUAT-INDUCED NEUROTOXICITY IN ZEBRAFISH (*Danio rerio*)

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ADVISOR: Vania Lucia Loro

CO-ADVISOR: Denis Broock Rosemberg

Considering the sodium selenite (Na_2SeO_3) antioxidant properties and the involvement of oxidative and nitrosative stress events in neurotoxicity induced by paraquat (PQ) herbicide, this study investigated the potential protective effect of a Na_2SeO_3 diet on biochemical and behavioral parameters in zebrafish exposed to PQ. The fish were pretreated with a Na_2SeO_3 supplemented diet for 21 days and then, the PQ treatment (20 mg/kg) to induction of neurotoxicity model was intraperitoneally administered in a total of 6 injections for 16 days (one injection every 3 days). Among behavioral parameters analyzed, Na_2SeO_3 pretreatment attenuated locomotor damage as decrease of distance traveled, the increase in time spent on top area of the tank and the increase in the latency to fish to enter in the closer to conspecifics area (interaction group).. In addition, the Na_2SeO_3 pretreatment abolished the exacerbation of freezing behavior and even prevented agonistic behavior like anxiety and aggression. Regarding to oxidative damage, Na_2SeO_3 pretreatment prevented the increase in carbonylation of protein (CP), reactive oxygen species (ROS) and nitrite/nitrate NOx levels caused by PQ treatment. Na_2SeO_3 pretreatment also prevented the increase in antioxidant enzymes catalase (CAT), glutathione peroxidase (GPx) and decrease in non-protein thiols levels (NPSH) induced by PQ treatment. The activation of the glutathione-S-tranferase (GST) enzyme by Na_2SeO_3 diet and PQ treatment was effective in preventing changes in levels of thiobarbituric reactive substances (TBARS). In conclusion, Na_2SeO_3 diet prevented behavioral changes and biochemical damage observed in PQ-treateds animals. Na_2SeO_3 pretreatment was beneficial because acts not only modulating the redox parameters, but also modulating phenotypes like anxiety and aggression in zebrafish PQ-treated. This study demonstrated for the first time that the zebrafish is an convenient organism model for "screening" of potential neuroprotective molecules against the neurotoxicity induced by PQ.

Key-words: Selenium, Fish, Herbicide, Behavior, Parkinson’s disease

LISTA DE ABREVIATURAS

INTRODUÇÃO

AO - Antioxidante
CAT - Catalase
DP - Doença de Parkinson
GPx - Glutationa peroxidase
GSH - Glutationa
Na_2SeO_3 - Selenito de sódio
ND - Neurônios dopaminérgicos
NO- Óxido nítrico
O_2•- - Radical superóxido
PQ – Paraquat (1,1'-dimetil-4,4'-bipiridina-dicloreto)
PQ^+ - Paraquat estado monovalente
PQ^{2+} - Paraquat estado divalente
ERNS - Espécies reativas de nitrogênio
EROS - Espécies reativas de oxigênio
Se - Selênio
SOD - Superóxido dismutase
TH – Tirosina hidroxilase

MANUSCRITO

BCIP - 5-bromo-4-chloro-3-indolyl phosphate
CAT- Catalase
CDNB - 1-chloro-2,4-dinitrobenzene
CNS - Central nervous system
DCF - dichlorofluorescein
DCFDA - 2,7-dichlorofluorescein-diacetate
DCFH-DA - 2′,7′-dichlorofluoresceindiacetate
DN - Dopaminergic neurons
DTNB - 5,5-dithio-bis-2-nitrobenzoic acid
EDTA - Sodium dodecyl sulfate
GPx - Glutathione reductase
GR - Glutatione reductase
GSH - Glutathione
GST - Glutathione-S-transferase
H_2O_2 - Hydrogen peroxide
MDA - Malondialdehyde
Na_2SeO_3 - Sodium selenite
NBT - Nitro blue tetrazolium
NO• - Nitric oxide
NOx - Nitrite/nitrate
NPSH - Non-protein thiols
$O2^{\bullet -}$ - Superoxide radical
$OH^{\bullet -}$ - Hydroxyl radical
$ONOO^-$ - Peroxynitrite radical
CP - Carbonyl protein
PD - Parkinson's disease

PQ - Paraquat
PQ^{+} - Paraquat monocation
PQ^{2+} - Paraquat dication
ROS - Rective oxygen species
RNS - Reactive nitrogen species
Se - Selenium
SOD - Superoxide dismutase
TBA - Thiobarbituric acid
TBARS - Thiobarbituric acid-reactive substance
TCA - Trichloroacetic acid
TH - Tirosyne hydroxylase
zMAO - Monoaminoxidase
GSSG - Oxidized glutathione
GSH - Reduced glutathione

SUMÁRIO

1. APRESENTAÇÃO

Esta dissertação está estruturada na seguinte forma: primeiramente são apresentados a **introdução** como uma revisão da literatura sobre os temas abordados na dissertação e os **objetivos** do estudo. A seguir, a **metodologia, resultados e discussão** são apresentados no item **manuscrito**. O item **conclusão** encontrado no final desta dissertação, apresenta interpretações gerais sobre o manuscrito contido nesta dissertação. O item **perspectivas** apresenta possibilidades de novos estudos a partir dos resultados obtidos. As **referências bibliográficas** apresentadas no final da dissertação referem-se às citações que aparecem no item introdução.

2. INTRODUÇÃO

Visando o aumento da produtividade, as atividades agrícolas em geral tem utilizado uma carga cada vez maior de agroquímicos para combater pragas, plantas daninhas, fungos e bactérias (Primel et al., 2005). No entanto, os pesticidas nem sempre são seletivos para suas espécies-alvo pretendidas e os efeitos adversos a saúde podem ocorrer em espécies não-alvo, incluindo humanos. Evidências apontam que a exposição a pesticidas aumenta o risco de desenvolvimento de câncer e doenças neurodegenerativas, pois os mesmos agem como disruptores endócrinos alterando diferentes funções fisiológicas (Eskenaz et al., 2008; Franco et al., 2010; Jones e Miller 2008). Dentro da classe geral de agroquímicos, os herbicidas têm chamado a atenção devido a sua facilidade de lixiviação até corpos de água e representam mais de 50 % de todos os agrotóxicos usados nos Estados Unidos e na Europa (Tekel e Kovacicová 1993; Tomita e Beyruth 2002). Além disso, a agricultura brasileira tem crescido exponencialmente nos últimos anos, e hoje, o Brasil é maior consumidor mundial de agrotóxicos (ANVISA, 2012).

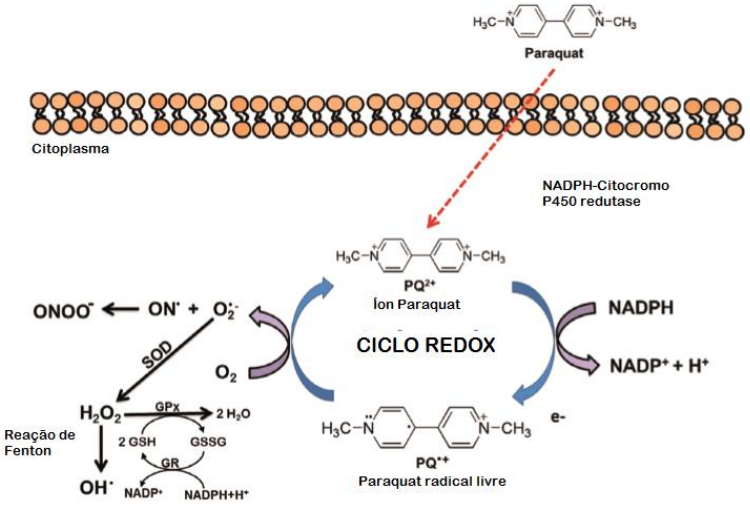
O paraquat (1,1'-dimetil-4,4'-bipiridina-dicloreto; PQ; Figura 1), conhecido comercialmente como Gramoxone®, é um herbicida não seletivo comumente utilizado na agricultura em culturas de fumo, arroz, café, entre outras, principalmente devido a seu baixo custo e rápida ação (Bromilow 2003). O PQ é um composto de amônio quaternário que pertence ao grupo químico bipiridílio e segundo a ANVISA (2007) é caracterizado como um herbicida que possui classificação toxicológica I (Extremamente Tóxico). Nas plantas, o PQ ocasiona prejuízos fisiológicos como a depleção de NADPH e inibição da fixação de CO_2, com consequente produção de radicais superóxidos, os quais promovem a destruição de membranas. Sua ingestão diária aceitável é de 0,004 mg/kg em humanos. O PQ também tem sido conhecido pelo seu uso para práticas de suicídio (Honoré et al., 1994).

Já foi demonstrado que a exposição ambiental ao PQ pode ter associação direta com o desenvolvimento de doenças neurodegenerativas como a doença de Parkinson (DP) (Dinis-Oliveira et al., 2006; Kamel et al., 2007; Tanner et al., 2011). A estrutura química do PQ é muito similar com a estrutura do MPP^+ (1-metil-4-fenil-piridínio), que é descrito como o metabólito ativo do MPTP (Dauer e Przedborski 2003). Assim como o MPTP (1-metil-4-fenil-1,2,3,6-tetraidropiridina), uma neurotoxina que provoca sintomas da doença da DP, o PQ pode entrar no sistema nervoso central (SNC) (Dinis-Oliveira et al., 2006) e causar a destruição seletiva dos neurônios dopaminérgicos (ND), assim como alterar o funcionamento de enzimas envolvidas na síntese ou degradação dopaminérgica, como a enzima tirosina

hidroxilase (TH), que também é utilizada como marcadora de morte neuronal dopaminérgica. É possível que a seletividade do PQ pelos ND aconteça por ele facilmente entrar nestas células através dos transportadores de dopamina (DATs), que o utilizam como um substrato. A entrada do PQ na célula auxiliada pelos DATs acontece quando o PQ^{2+} (estado divalente nativo) é transformado em PQ^{+} (estado monovalente). Assim, dentro da célula, o PQ irá gerar radicais superóxido e outras espécies reativas desencadeando a neurotoxicidade (McCormack et al., 2006; Rappold et al., 2011).

O PQ exerce os seus efeitos tóxicos devido a seu ciclo redox. Uma vez dentro da célula, o PQ atua a nível citosólico (Figura 2), mas também conduz a uma toxicidade mitocondrial indireta (Figura 3) (Blanco-Ayala et al., 2014). As enzimas capazes de iniciar o ciclo redox do PQ^{2+} estão presentes no microssoma, na membrana plasmática e em componentes citosólicos que incluem a NADPH-oxidase, óxido nítrico sintetase e NADPH-citocromo P450 redutase (Cochemé e Murphy 2008). No citosol, a toxicidade do PQ é mediada pela sua redução pela NADPH-citocromo P450 redutase ao radical monocátion PQ^{+}. Este radical reage espontaneamente com o oxigênio gerando o radical ânion superóxido (O_2•-) e regenerando o PQ^{2+} inicial, que pode ser submetido novamente ao ciclo de oxirredução ou permanecer no tecido por longos períodos de tempo (Czerniczyniec et al., 2011; Takizawa et al., 2007). A oxidação de NADPH é contínua e isso conduz a uma deficiência na reciclagem da glutationa (GSH), prejudicando a atividade de vários sistemas antioxidantes (Franco et al., 2009).

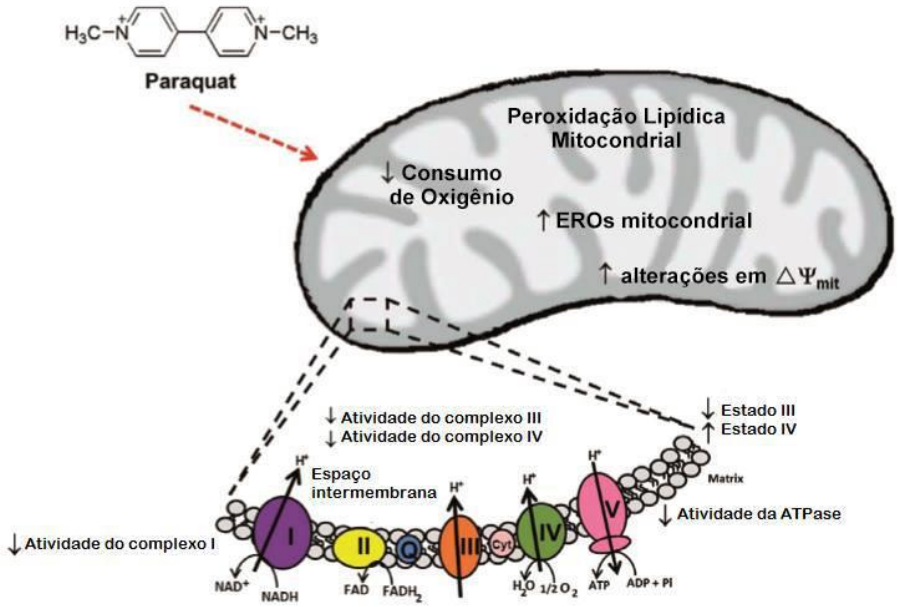
Figura 1 – Mecanismo de toxicidade do PQ na matriz mitocondrial



Fonte: Adaptado de Blanco-Ayala et al., 2014

Estudos recentes tem demonstrado que a mitocôndria é um componente-chave no mecanismo pelo qual o PQ exerce toxicidade e conduz a morte celular (Blanco-Ayala et al., 2014; Castello et al., 2007; Cochemé e Murphy 2008; Drechsel e Patel 2009). O PQ pode agir aumentando a peroxidação lipídica, gerando espécies reativas de oxigênio (ERO) dentro da mitocôndria. Esses efeitos podem alterar diversos parâmetros na bioenergética mitocondrial como: consumo de oxigênio, a atividade do complexo I (Cochemé e Murphy 2009), o potencial de membrana, e ainda, o PQ e é capaz de inibir parcialmente a atividade da enzima ATP sintase conduzindo a diminuição na síntese de ATP (Palmeira et al., 1995). O PQ também causa um aumento concentração-dependente no estágio IV da respiração mitocondrial (Kopaczyk-Locke 1977) e efeitos inibitórios no estágio III (Rossouw 1978).

Figura 2 – Mecanismos de toxicidade do PQ na mitocôndria



Fonte: Adaptado de Blanco-Ayala et al., 2014 (Δψ mit: potencial de membrana mitocondrial)

O estudo de propriedades neurotóxicas do PQ vem trazendo informações valiosas sobre os possíveis mecanismos envolvidos na progressão da neurodegeneração associada com a toxicidade ambiental (Franco et al., 2009). Recentemente, o PQ tem se mostrado um eficiente modelo de neurotoxicidade que assemelha algumas características da DP (Blesa et al., 2012; Le et al., 2014; Shimizu et al., 2003). Estudos têm explorado essas ações em vários modelos experimentais, desde linhagens celulares, *Drosophila melanogaster*, *Caenorhabditis elegans*, até modelos vertebrados como peixe-zebra e roedores (Ellwanger et al., 2015; Jahromi et al., 2015; Lima et al., 2014; Nunes et al., 2016; Wang et al., 2015). Estudos já

demonstraram que uma exposição crônica a esse herbicida em peixe-zebra produz além de condições de estresse oxidativo e citotoxicidade, uma diminuição do número de ND no encéfalo, alterações nos dos níveis de dopamina e seus metabólitos, assim como alterações na atividade da enzima TH e na imunorreatividade do transportador de dopamina (Bortolotto et al., 2014; Bretaud et al., 2004; Izumi et al., 2014).

Levando em consideração que a neurotoxicidade induzida pelo PQ esta em grande parte associada a condições de estresse oxidativo e nitrosativo, e que essa condição pode vir a desencadear doenças neurodegenerativas como a DP, a busca por compostos que atuem prevenindo ou amenizando os efeitos deletérios da exposição ao PQ vem aumentando. Nesse contexto, os antioxidantes (AO) têm emergido como potenciais agentes terapêuticos para prevenir a lesão celular induzida por PQ. Os AO podem agir diminuindo danos oxidativos em nível de DNA, lipídios e proteínas, além de prevenir a depleção de sistemas antioxidantes, condições de inflamação, edema e morte celular (Blanco-Ayala et al., 2014; Suntres 2002).

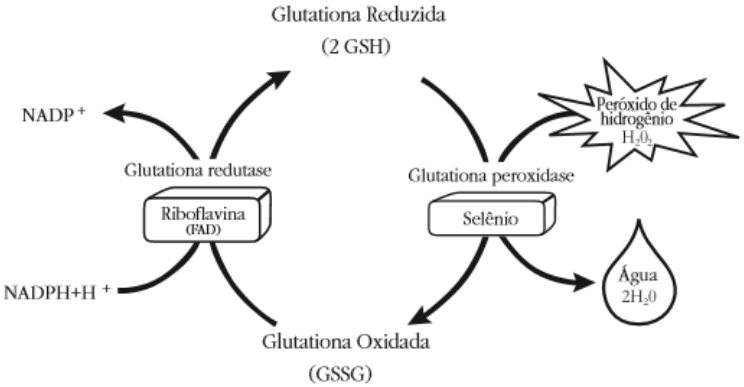
As ERO são moléculas eletrofílicas fisiologicamente produzidas, que podem reagir com os lipídeos, proteínas e ácidos nucleicos e resultar em danos oxidativos quando produzidas em altas concentrações. ERO também podem reagir com o óxido nítrico (NO), formando as espécies reativas de nitrogênio (ERN), que são igualmente prejudiciais (Cadenas et al., 2000). Ambas as espécies são geralmente eliminadas pelas defesas enzimáticas celulares e compostos antioxidantes não enzimáticos, mantendo o estado redox natural da célula. No entanto, em situações em que há um desequilíbrio entre a produção de ROS/RNS e as defesas antioxidantes naturais, ocorre o estresse oxidativo (Pisoschi e Pop 2015).

Nos últimos anos, as propriedades antioxidantes do mineral selênio (Se) vêm se destacando na pesquisa biomédica (Cardoso et al., 2015; Letavayová et al., 2008). O Se foi descoberto em 1817 pelo químico sueco Jöns Jacob Berzelius (Boyd 2011) e é ricamente encontrado em grãos, cereais e carnes. Representa o elemento 34 da tabela periódica e caracteriza-se como um semi-metal. Durante muito tempo, o Se foi conhecido pela sua toxicidade e só em 1957 teve sua importância reconhecida para o metabolismo celular (McLaren 1999; Mehdi et al., 2013). O Se desempenha um papel importante para o adequado funcionamento de todas as células e é um micronutriente essencial para manter o funcionamento cerebral normal em humanos e outros vertebrados (Pillai et al., 2014; Rayman 2000; Steinbrenner e Sies 2009; Whanger 2016).Vários estudos têm demonstrado que este mineral influencia em patologias que afetam o sistema nervoso central (Benton et al., 2002; Sharar et al., 2010).

Nesse contexto, uma suplementação com o antioxidante Se poderia prevenir os efeitos deletérios causados pelo PQ, assim como proporcionar uma melhor condição de saúde aos indivíduos, tornando-os menos vulneráveis aos efeitos desse herbicida. Apesar de ainda não ser possível descrever exatamente o mecanismo pelo qual o PQ exerce sua toxicidade, o uso do Se como uma estratégia para prevenir tais danos é plausível já que possui ação antioxidante e neuroprotetora, e o estresse oxidativo é uma das principais causas da toxicidade celular e morte neuronal desencadeada pelo PQ (Liu et al., 2013; Maldonado et al., 2012; Wang et al., 2014).

As funções biológicas do Se e seu envolvimento em processos patológicos são mediadas em parte pelas selenoproteínas como as enzimas glutationa peroxidase, tiorredoxina redutase e iodotironina redutase, as quais contem selenocisteína em seu sitio ativo (Mehdi et al., 2013). As selenoproteinas desempenham atividades variadas, como o transporte de Se (Burk e Hill 2009), imunomodulação, regulação da apoptose (Roman et al., 2014) e controle da estado redox celular (Arigony et al., 2013). Sob a forma dessas selenoenzimas o Se também está envolvido na proteção de células neuronais contra o estresse oxidativo (Stenbrenner e Sies 2009). A família das enzimas glutationa peroxidases (GPxs) é a mais conhecida, sendo que essas enzimas são dependentes de Se e atuam protegendo as membranas lipídicas e macromoléculas do dano oxidativo, reduzindo peróxido de hidrogênio e hidroperóxidos a partir da glutationa reduzida (GSH) (Figura 3) (Tapiero et al., 2003). As enzimas da classe das GPxs também são relacionadas com a deficiência nutricional de Se (Mehdi et al., 2013, Pillai et al., 2014).

Figura 3 – Papel do selênio no ciclo da glutationa



Fonte: Cominetti et al., 2011

A faixa de concentração em que os compostos de Se desempenham funções celulares essenciais ou exercem efeitos tóxicos é estreita (Rayman 2000). Entretanto, quando usado em doses adequadas, o Se mostra efeitos antioxidantes e neuroprotetores por atuar diretamente no *scavenging* de radicais livres pela redução de hidroperóxidos e lipoperóxidos (Li et al., 2014; Solovyev 2015). Estudos recentes têm demonstrado que o Se pode atuar reduzindo a geração de ROS, peroxidação de lipídeos e alterações no sistema de defesa AO induzida por xenobióticos (Menezes et al., 2012; Nogueira e Rocha 2010). O Se também participa na regulação de processos inflamatórios e respostas imunes, assim como na regulação da atividade tumoral e dos hormônios da tireoide (Köhrle et al. 2000). Além disso, evidências apontam que uma suplementação com Se pode prevenir déficits cognitivos, desordens de humor e restaurar déficits funcionais encontrados em diversas doenças neurodegenerativas (Adebayo et al,. 2014; Adebayo et al., 2016; Benton 2002; van Eersel et al., 2010; Chen e Berry 2003).

O selenito de sódio (Na_2SeO_3) é uma forma inorgânica de Se que é variavelmente absorvida até níveis de 50-90% (Pillai et al., 2014). Uma cascata de reações complexas pode converter os compostos de Se inorgânicos tais como o Na_2SeO_3 em formas orgânicas e vice-versa. Assim, o Na_2SeO_3 é transformado em seleneto pela via glutationa-glutaredoxina e tioredoxina antes de ser incorporado nas selenoproteínas. Um estudo realizado recentemente demonstrou que o uso terapêutico do Na_2SeO_3 em modelo de toxicidade por induzido por PQ contribuiu para a manutenção da atividade locomotora e integridade do DNA em encéfalo de roedores (Ellwanger et al., 2015). O uso do Na_2SeO_3 demonstrou também prevenir a inibição das enzimas antioxidantes catalase (CAT), superóxido dismutase (SOD) e GPx, além de diminuir a peroxidação lipídica em peixes-zebra expostos ao cádmio (Betancor et al., 2015).

O desenvolvimento de novos modelos experimentais tem se mostrado de grande importância no meio científico. Dentre esses modelos animais, destaca-se o peixe *Danio rerio*, também conhecido por peixe-zebra ou “paulistinha”, um pequeno teleósteo de 3-4 centímetros, pertencente à família Cyprinidae (Whitlock e Westerfield 2000). Do ponto de vista genético, o peixe-zebra tornou-se atrativo por ser utilizado como intermediário entre os invertebrados (*Drosophila melanogaster* e *Caenorhabditis elegans*) e os roedores (Guo 2004; Ninkovic e Bally-Cuif 2006). A grande maioria dos genes descobertos nesta espécie são evolutivamente conservados e homólogos aos mamíferos (Parng et al., 2002). O grau de homologia com humanos é de 70-80% (Barbazuk et al., 2000), permitindo a extrapolação dos resultados encontrados nesta espécie em relação a humanos de maneira mais direta do que aqueles obtidos em invertebrados (Ninkovic e Bally-Cuif 2006) e de forma mais econômica

em relação a roedores (Goldsmith 2004). Pode-se destacar, com isso, que o peixe-zebra é um animal que combina a relevância de ser um vertebrado com a escala de um invertebrado.

O peixe-zebra também possui as principais estruturas encefálicas, neurotransmissores, receptores e hormônios dos mamíferos (Guo 2004, Panula et al., 2006). Muitos sistemas de neurotransmissão já foram identificados nesta espécie, tais como: glutamatérgico (Edwards e Michel 2002), colinérgico (Behra et al., 2002), dopaminérgico (Boehmler et al., 2004), serotoninérgico (Rink e Guo 2004), purinérgico (Kucenas et al., 2003; Rico et al., 2003) e gabaérgico (Kim et al., 2004).

Recentemente também foram desenvolvidos estudos avaliando características comportamentais do peixe-zebra (Blaser e Rosemberg 2012; Cachat et al., 2010; Maximino et al., 2010; Piato et al., 2011). O peixe-zebra adulto apresenta um repertório comportamental bastante complexo, onde a exposição a agentes estressores pode evocar medo ou comportamento tipo-ansiedade facilmente quantificáveis através de exploração reduzida, aumento da escototaxia (aversão a ambientes claros), geotaxia (resposta de mergulho), tigmotaxia (preferência pela periferia do tanque), congelamento (imobilidade junto a aumento da frequência respiratória), avaliação de risco (entrada parcial no compartimento claro e rápido retorno para o compartimento escuro), movimentos erráticos (repentinas mudanças bruscas de velocidade e direção do nado), preferência por coespecíficos e/ou agressividade (Egan et al., 2009; Kalueff et al., 2013). Atributos práticos como fácil manejo, pequeno espaço requerido, baixo custo de manutenção, alta reprodutividade e baixo custo para triagens em larga escala (Littleton e Hove 2013) fazem esse com que o peixe-zebra seja atraente para estudos de laboratório. Nesse sentido, a utilização do peixe-zebra para pesquisa em neurociências, bioquímica, toxicologia e biologia do comportamento tem crescido muito na última década (Fetcho 2007; Kalueff et al., 2015; Rico et al., 2011).

3. OBJETIVOS

3.1 Objetivo geral:

Investigar o efeito protetor do selenito de sódio (Na_2SeO_3) sobre parâmetros bioquímicos e comportamentais em um modelo de neurotoxicidade induzido por PQ em peixe-zebra (*D. rerio*).

3.2 Objetivos específicos:

- Analisar através de testes comportamentais: parâmetros motores (locomoção, exploração e motricidade) e não-motores (comportamentos do tipo ansiedade) em peixes-zebra alimentados com Na_2SeO_3 e/ou tratados com PQ;

- Determinar a atividade da enzima monoaminoxidase e a expressão da enzima tirosina hidroxilase em encéfalo de peixes-zebra alimentados com Na_2SeO_3 e/ou tratados com PQ;

- Determinar o perfil oxidativo, nitrosativo e resposta antioxidante em encéfalo de peixes-zebra alimentados com Na_2SeO_3 e/ou tratados com PQ;

4. MANUSCRITO

Sodium Selenite Prevents Paraquat-Induced Neurotoxicity in Zebrafish

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Abstract

Considering the antioxidants properties of sodium selenite (Na_2SeO_3) and the involvement of oxidative stress events in paraquat-induced neurotoxicity, this study investigated the potential protective effect of dietary Na_2SeO_3 on biochemical and behavioral parameters of zebrafish exposed to paraquat (PQ). Fish were pretreated with Na_2SeO_3 diet for 21 days and then PQ (20 mg/kg) was administered intraperitoneally with six injections for 16 days. Na_2SeO_3 diet prevented the decrease in locomotor impairments, the increased in time spent in top of tank and the increase in latency to enter the area closer to conspecifics caused by PQ treatment. Moreover, Na_2SeO_3 dietary abolished the exacerbation of freezing and prevented the agonistic behavioral as aggression detected in PQ treateds. Na_2SeO_3 pretreatment prevented the increase of protein carbonyl (CP), reactive oxygen species (ROS) and nitrite/nitrate (NOx) levels, as well as prevented the increase in catalase (CAT) and glutathione peroxidase (GPx) activities caused by PQ treatment. Additionally, Na_2SeO_3 pretreatment prevents the decreased in non-protein thiols (NPSH) levels induced by PQ treatment. In conclusion, dietary Na_2SeO_3 improves behavioral and biochemical function impaired by PQ treatment in zebrafish, by modulating not only redox parameters, but also anxiety- and aggressive-like phenotypes in zebrafish. We also demonstrate that zebrafish is a convenient model organism for screening of potential neuroprotective molecules.

Key-words: Selenium, Fish, Herbicide, Parkinson's disease, Behavioral

1. Introduction

Paraquat (PQ) (1,1′-dimethyl-4,4′-bipyridinium dichloride) is a highly toxic quaternary nitrogen herbicide widely used in agriculture due to its low cost and rapid action (Dinis-Oliveira et al. 2006). In animals, PQ acts mainly on dopaminergic neurons (DN) due to its specificity with the same neutral amino acid transporter used by L-valine and L-dopa (Manning-Bog et al. 2003). DN are sensitive to oxidative stress, which is one of the main causes of dopaminergic neural death (McCormack et al. 2006; Rappold et al. 2011).

PQ operates in cytosolic level, but also leads to an indirect mitochondrial toxicity (Blanco-Ayala et al. 2014). PQ^{2+} (radical dication) is reduced by NADPH-cytochrome P450 reductase to radical monocation $PQ•^{+}$. This radical reacts spontaneously with oxygen generating superoxide ($O2•^{-}$) and regenerating the initial PQ^{2+}. Superoxide dismutases (SOD) dismutate $O2•^{-}$ to hydrogen peroxide (H_2O_2), which is further metabolized by gluthatione peroxidase (GPx) and catalase (CAT). $O2•^{-}$ also reacts with nitric oxide (•NO) leading to the formation of peroxynitrite ($ONOO^{-}$). H_2O_2 can also react with myeloperoxidases to produce hypochlorous acid or can be reduced to hydroxyl radical ($OH•^{-}$) through Fenton reactions (Czerniczyniec et al. 2011; Franco et al. 2010; Takizawa et al. 2007). PQ also increases reactive oxygen species (ROS) formation in mitochondria (Castello et al. 2007; Cochemé and Murphy 2008; Czerniczyniec et al. 2011). These effects can change various parameters in terms of bioenergetics, such as oxygen consumption, electron transport chain activity, membrane potential, and consecutively impair enzyme ATP synthase activity (Blanco-Ayala et al. 2014; Drechsel and Patel 2009).

ROS produced by PQ redox cycle can react with cell membranes causing NADPH oxidation, impaired recycling of oxidized glutathione, and lipid peroxidation, contributing to oxidative damage (Franco et al. 2009; Suntres 2002). PQ is considered a potential environmental neurotoxin that has been associated with increased risk for neurodegenerative diseases, such as Parkinson disease (PD) (Dinis-Oliveira et al. 2006; Wang 2013). In addition, PQ has been used as a neurochemical model to mimic to parkinsonism-related phenotypes in non-human animals, getting valuable information regarding the potential mechanisms involved in the progression of neurodegeneration (Blesa et al. 2012; Le et al. 2014; Shimizu et al. 2003).

Antioxidants have emerged as potential therapeutics for the treatment of neurodegenerative diseases, where the oxidative stress is directly involved (Pisoschi and Pop 2015). Selenium (Se) is an essential micronutrient for maintaining normal brain function in

humans and other vertebrates that has received attention for its strong antioxidant properties (Pillai et al. 2014; Rayman 2000; Steinbrenner and Sies 2009; Whanger 2016). The biological functions of Se are mediated in part by selenoproteins, which contain selenocysteine in their active site, as well as by some antioxidant enzymes (e.g. glutathione peroxidase, thioredoxin reductase, and iodotironina reductase) (Cardoso et al. 2015). Sodium selenite (Na_2SeO_3) is an inorganic form of Se variably absorbed (up to 50-90%) and it is converted to selenide via glutathione-glutaredoxin and thioredoxin pathways before incorporation into selenoproteins (Navarro-Alarcon and Cabrera-Vique 2008).

Although the concentration range associated with Se nutritive requirements and toxicity is narrow (Rayman 2000). Se shows antioxidant and neuroprotective effects by scavenging free oxygen radicals, protects DNA, lipids and proteins by reducing hydroperoxides and lipoperoxidases when used in appropriate levels (Li et al. 2014; Solovyev 2015). Se is able to regulate inflammation, immune responses, antitumor activity and thyroid hormones in animals (Köhrle et al. 2009). A large body of evidence also pointed that Se supplementation can prevent cognitive decline, mood disorders and restore functional deficits in several neurodegenerative diseases (Adebayo et al. 2014; Adebayo et al. 2016; Benton 2002; Chen and Berry 2003; van Eersel et al. 2010).

New experimental models have emerged in recent years, including the zebrafish (*Danio rerio*) that has been widely used in toxicology and behavioral neuroscience studies, being considered a promising effective organism for modelling neurodegenerative disease-related phenotypes, like PD (Best and Alderton 2008; Esch et al. 2012; Makhija and Jagtap 2014). Despite the evident anatomic differences between the central nervous system (CNS) structures of teleosts and mammals, the neurotransmitter systems in zebrafish are evolutionary conserved. This feature allows the basic research of the molecular mechanisms underlying drug effects, neurological diseases or even facilitates the validation of several psychopathology models (Kalueff et al. 2015; Panula et al. 2006; Rico et al. 2011). Considering the promising antioxidants properties of Se and the involvement of oxidative stress events in PQ-induced neurotoxicity, the aim of this study was to investigate whether dietary Na_2SeO_3 prevents the biochemical and behavioral parameters triggered by PQ in zebrafish.

2. Materials and Methods

2.1 Animals

Adult zebrafish (4-6 months-old) of heterogeneous wild-type stock (standard short-fin phenotype) of both sexes, weighing 0.6 ± 0.1 g and measuring 3.0 ± 1.0 cm of length, were obtained from a local commercial distributor (Hobby Aquários, RS, Brazil). Fish were acclimatized in tank (40 L) with partitions filled with non-chlorinated water treated with AquaSafeTM (Tetra, VA, USA) under constant aeration, temperature-regulated (27 ± 1 ºC) and photoperiod cycle of 14/10 hours. This tank with partitions allowed the maintenance of 16 animals individually separated in the same aquarium. Additionally, they were able to maintain direct visual contact with other fish, minimizing the stress of isolation and permitting a precise identification of each experimental subject (Nunes et al. 2016). All animals were experimentally naïve and fed with alcon BASIC^{TM} flakes (Alcon, Brazil) twice daily. The animal experimentation fully adhered to the National Institute of Health Guide for Care and Use of Laboratory and the protocols were approved by the Ethics Commission on Animal Use of the Federal University of Santa Maria (protocol number 1777051115).

2.2 Experimental Design and PQ Administration

Initially, fish were divided into two groups and fed with two diets for 21 days: fish non-supplemented with Na_2SeO_3 (n= 150) and fish supplemented with Na_2SeO_3 (n= 150). After pre-feeding period, fish were subdivided in four experimental groups (n=75 per group): control group (CT) [fish fed with diet without Na_2SeO_3 and treated with saline], paraquat group (PQ treatment) [fish fed with diet without Na_2SeO_3 and treated with PQ], Na_2SeO_3 group (SE diet) [fish fed a diet containing Na_2SeO_3 and treated with saline] and selenium + paraquat group (SE+PQ) group [fish fed a diet containing Na_2SeO_3 and treated with PQ]. PQ (methyl viologen dichloride hydrate, Sigma®) at 20 mg/kg was diluted into 0.9 % saline solution and administrated intraperitoneally (i.p.). It was applied one injection every 3 days for 16 days, with a total of six injections (Bortolotto et al. 2014; Nunes et al. 2016). Each injection was administered in a volume of 10 µL after fish being anesthetized with 0.1 g/L tricaine (3-amino benzoic acidethylester) according Wilson et al. (2009). During the PQ injections period, all fishes fed only with control diet.

2.3 Diet Preparation

The diet composition was based on studies of our research group (Menezes et al. 2014; 2016) and Na_2SeO_3 was used at 1 mg/kg. This concentration was based on previous data, which demonstrated that 1.0 - 3.0 mg/kg of Na_2SeO_3 did not cause signals of toxicity in zebrafish (Banni et al. 2011, Hamilton, 2003). Briefly, to obtain the diets containing Na_2SeO_3, the respective compound was added to control diet, and all the ingredients were completely mixed before further homogenization. In this experimental protocol, fish were fed 3 % biomass per day (Menezes et al. 2014; 2016) divided into two equal meals at 9:00 and 16:00 hours.

2.4 Behavioral Measurements

The behavioral tests were performed twenty-four hours after the last PQ injection between 10:00 am and 4:00 pm and a number of 12-14 animals was used per experimental group. The apparatuses (15 × 10 × 25 cm, height × depth × length) were filled with 2 L of nonchlorinated water (27 ± 1 ºC) and the experimental procedures were performed on a stable surface with all environmental distractions kept to a minimum. The behavioral of zebrafish was recorded using a webcam connected to a laptop at a rate of 30 frames/s using appropriate video-tracking software (ANY-maze^{TM}, Stoelting CO, USA).

2.4.1 Novel tank test

Locomotor and exploratory activities were analyzed in novel tank test, which may reflect habituation to novelty stress (Cachat et al. 2010; Mezzomo et al. 2016; Rosemberg et al. 2011). After the treatment period, zebrafish were individually placed in a novel apparatus filled with 2 L water and their swimming behavioral was recorded. The apparatus was virtually divided into two horizontal sections (bottom and top) to assess the vertical exploration by the following endpoints: number of entries and time spent in bottom or top area. Distance travelled and absolute turn angle were used to measure motor pattern. Fear/anxiety-related behavioral were determined of the number and duration of freezing episodes. Freezing was computed when zebrafish were immobile presenting increased opercular movements for at least 2 s. The test was carried out for 6 minutes (min) (Gerlai et al. 2000).

2.4.2 Preference by conspecifics

The preference by conspecifics reflects a natural tendency of zebrafish to establish an interaction group (Gerlai et al. 2000; Saverino and Gerlai 2008). The aggregation of fish and formation of a shoal can be modified by different compounds. Initially, the animal was placed individually on the central aquarium, situated between an empty tank and another tank with five conspecifics (stimulus group). The apparatus was divided into two sections and A1 represented the area closer to stimulus group. The time in which fish remained close to each tank was measured and expressed as a tendency of group approach. The test was performed for 2 min.

2.4.3 Aggression test

The aggression test was performed using the inclined mirror-image stimulus. Fish were individually placed into to experimental tank and a mirror was placed inclined at 22.5° to the back wall of the tank so that the left vertical edge of the mirror was touching the side of the tank and the right edge was further away. Thus, when the experimental fish swam to the left side of the tank their mirror image appeared closer to them. Fish were able to explore both compartments for 5 min and the following behaviors were determined: number of entries and time spent (s) in each section (A1, A2) and number and duration (s) of attacks. Entry to the left segment (A1) indicated preference for proximity to the “opponent”, whereas entry to the right segment (A2) implied avoidance. In addition, the amount of time the fish spent with aggressive display, or attack behavioral, was also measured and analyzed as aggression. Aggressive display was defined as a posture during in which the fish erects its dorsal, caudal, pectoral, and anal fins, usually associated with undulating body movements and attacks. Attack behavioral is a characteristic short bout of fast swimming directed towards the opponent when fish open the mouth and bite the image (Fontana et al. 2016; Gerlai et al. 2000).

2.5 Biochemical analysis

2.5.1 Tissue preparation

Twenty-four hours after the last PQ injection, zebrafish were anesthetized with 0.25 g/L tricaine (Wilson et al. 2009) and euthanized by punching the spinal cord behind the opercula and brains were dissected out in ice. To perform the biochemical analyses (except zMAO activity and TH expression) the brains were homogenized in a proportion of 1 brain to

150 µL Tris-HCl 50 mM, pH 7.4 buffer, centrifuged (3000 *g* for 10 min, - 4º C) and the supernatant was transferred to microtubes and kept at - 80 ºC for posteriors assays. All experiments were performed in duplicate.

2.5.2 Analyses of zMAO activity and TH expression

The enzyme activity of monoaminoxidase (zMAO) was determined based on protocols previously described (Krajl 1965; Matsumoto et al. 1984). Two zebrafish brains were pooled and homogenized in 500 µL of buffer solution containing 16.8 mM Na_2HPO_4 and 10.6 mM KH_2PO_4 isotonized with sacarose, pH 7.4. Samples were centrifuged at 1000 *g* for 5 min and the supernatants were removed. An aliquot of 100 µl (approximately 120 µg protein) was mixed to 460 µL of assay buffer (168 mM Na_2HPO_4 and 10.6 mM KH_2PO_4, isotonized with KCl, pH 7.4) and preincubated at 37°C for 5 min. The reaction was started with the addition of kynuramine dihydrobromide at a saturating concentration of 110 µM (Aldeco et al. 2011; Maximino et al. 2013) in a total volume of 700 µL. After 30 min incubation, the reaction was terminated by adding 300 µL of 10 % trichloroacetic acid (TCA) and the tubes were kept on ice. Incubation time and protein concentration were chosen in order to ensure the linearity of the reactions. Samples were further centrifuged at 16.000 *g* for 5 min and 800 µL of supernatant was mixed to 1 mL of 1M NaOH. The fluorescence intensity was measured spectrofluorimetrically with excitation 315 nm and emission 380 nm. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-OH quinoline. Specific activity was expressed as nmol 4-OH quinoline/min/mg protein.

For determination of tyrosine hydroxylase (TH) expression two brains from adult fish were homogenized in 250 µL of lysis buffer (4 % sodium dodecyl sulfate, 2 mM EDTA, 50 mM Tris, 0.5 mM Na_2VO_4, 2 µg/mL aprotinin, 0.1 mM benzamidine, 0.1 mM PMSF). Samples were boiled for 6 min and centrifuged at 8000 g at 4 °C for 10 min. The supernatant was used to determine protein concentration using the Lowry method (Lowry et al.1951). Then, the samples (50 µg) were mixed with 10 % glycerol and 8 % 2-mercaptoethanol and resolved by 10 % SDS-PAGE. The samples were transferred into a nitrocellulose membrane (Millipore, USA). Proteins on the membrane were stained with a Ponceau solution as a loading control (Romero-Calvo et al. 2010). Membranes were then blocked with 1 % bovine serum albumin and incubated overnight with an anti-TH (1:10.000; Millipore; AB152) and after with alkaline phosphatase-coupled secondary antibody for 1 h (1:10.000; Millipore). The reaction was determined by a colorimetric assay using nitro blue tetrazolium (NBT)/5-bromo-4-chloro-3-indolyl phosphate (BCIP) as a substrate (de Freitas et al. 2016). Finally, all values

were normalized using Ponceau quantification an expressed as TH immunoreactivity relative optical density.

2.5.3 Biomarkers of oxidative and nitrosative damage

Lipid peroxidation was estimated by thiobarbituric acid-reactive substance (TBARS) production, which is widely performed for measurement of lipid redox state (Draper and Hadley 1990). Briefly, 80 µL of homogenate brain (80-100 µg protein) were mixed with 160 µL of 10% TCA and centrifuged (10.000 *g*, 10 min). Supernatants (100 µL) were mixed with 100 µL of 0.67% thiobarbituric acid (TBA, 4,6-Dihydroxypyrimidine-2- thiol) and heated at 100 °C for 30 min. TBARS levels were determined by the absorbance at 532 nm using malondialdehyde (MDA) reaction with TBA in microplate reader. MDA was used as standard and results were expressed as nmol MDA/mg protein.

The levels of carbonylated protein (CP) were assayed based on the method described by Yan et al (1995). Briefly, soluble protein (200 µL) was reacted with 10 mM DNPH in 2 N hydrochloric acid. After incubation at room temperature for 1 h in the dark, 0.15 mL of denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, containing SDS 3.0 %), 0.5 mL of heptane (99.5 %), and 0.5 mL of ethanol (99.8 %) were added sequentially, kept in continuous agitation for 40 s, and centrifuged for 15 min at 3000 *g*. Then, the protein isolated from the interface was washed twice by resuspension in ethanol/ethyl acetate (1:1) and suspended in 0.25 mL of denaturing buffer. CP content was measured spectrophotometrically at 370 nm in microplate reader. The total carbonylation was expressed as nmol carbonyl/mg protein and calculated using a molar extinction coefficient of 22.000 M/cm.

Reactive oxygen species (ROS) levels were measured using the fluorescent dye 2,7-dichlorofluorescein-diacetate (DCFDA) as described by Ali et al. (1992). One aliquot of 10 µL homogenate were mixed with 10 µL of 0.1 mM 2′,7′-dichlorofluoresceindiacetate (DCFH-DA) and 130 µL of Tris HCl buffer. ROS levels were determined by fluorescence at emission (570 nm) and excitation (545 nm) using dichlorofluorescein (DCF) as standard. Results were expressed as µmol DCF/mg protein.

For nitrite/nitrate (NOx) levels determination, 200 µl of brain homogenate was mixed into 200 mM Zn_2SO_4 in the presence of acetonitrile (96 %, HPLC grade). Then, the homogenate was centrifuged at 16.000 *g* for 20 min at 4ºC, and the supernatant was collected for analyses of NOx content as previously described (Miranda et al. 2001). Results were expressed as µmol NOx/mg protein.

2.5.4 Antioxidant parameters

Superoxide dismutase (SOD) activity was performed based on inhibition of the radical superoxide reaction with adrenalin as described by Misra and Fridovich (1972). A unit of SOD is defined as the amount of enzyme that inhibits by 50 % the speed of oxidation of adrenaline. SOD activity is determined by measuring the speed of adrenochrome formation, observed at 480 nm, in a reaction medium containing glycine NaOH (50 mM, pH 10), adrenaline (1 mM) and homogenate (20-30 µg of protein). The activity was assessed in a microplate reader and expressed as U SOD/mg protein.

Catalase (CAT) activity was measured by the rate of decrease in H_2O_2 absorbance at 240 nm by ultraviolet spectrophotometry (Aebi 1984). The assay mixture had 1 mL potassium phosphate buffer (50 mM, pH 7.0), 0.05 mL H_2O_2 (0.3 M) and 0.01 mL homogenate (20-30 µg of protein). The results were expressed as µmol/min/mg protein.

Glutathione peroxidase (GPx) activity was assessed by ultraviolet spectrophotometry following the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase (Paglia and Valentine 1967). The assay mixture consisted of potassium phosphate buffer (100 mM, pH 7.0), 1 mM NaN_3, 1 mM reduced glutathione, 0.15 mM NADPH and homogenized with 20 µL tissue (40-60 µg of protein). The reaction was started by the addition of 30 µL 0.4 mM H_2O_2 totalizing a final volume of the reaction mixture of 300 µL in a microplate reader. The specific activity was expressed as nmol/min/mg of protein.

The determination of glutathione reductase (GR) activity was based on the consumption of NADPH at 340 nm. The assay mixture consisted of and homogenized with 20 µL tissue (40-60 µg of protein) and 250 µL of system (TFK 0.15 M and NADPH 0.15 mM). The reaction was started by the addition of 30 µL of GSSG (oxidized glutathione substrate totalizing a final volume of the reaction mixture of 300 µL in a microplate reader. The specific activity was determined using the extinction coefficient of 6.22 mM/cm and expressed as nmol/min/mg protein (Carlberg and Mannervik 1985).

Glutathione S-transferase (GST) activity was analyzed according to Habig et al. (1974). The assay mixture contained 1mM 1-chloro-2, 4-dinitrobenzene (CDNB) in ethanol, 10 mM glutathione reduced, 20 mM potassium phosphate buffer (pH 6.5), and 20 µl of the tissue homogenates (40-60 µg of protein). Enzyme activity was calculated by the changes in absorbance at 340 nm using a molar extinction coefficient of 9.6mM/cm in a microplate reader. One unit GST activity was defined as the amount of enzyme required to catalyze the

conjugate 1 mol CDNB with GSH/min at 25 ºC. The activity was expressed as µmol GS-DNB/min/mg protein.

To determine non-protein thiols levels (NPSH) an aliquot (100 µL) was mixed with 100 µL of 10 % TCA and centrifuged (3.000 *g*, 10 min at 4 ºC). Supernatants (60-80 µg of protein) were mixed with DTNB (5,5-dithio-bis-2-nitrobenzoic acid,0.01 M dissolved in ethanol) and the intense yellow color developed was measured at 412 nm after 1 h (Ellman 1959) in a microplate reader. Results were expressed as nmol SH/ mg protein.

2.5.5 Protein determination

Protein was determined by the Coomassie blue method using bovine serum albumin as standard. Absorbance of samples was measured at 595 nm (Bradford 1956).

2.6 Statistics

Normality of data and homogeneity of the variances were analyzed by Shapiro-Wilk and Kolmogorov-Smirnov tests, respectively. Data were expressed as mean ± standard error of the mean (S.E.M.) and analyzed by Two-Way ANOVA using with factors: diet and treatment, followed by the Student-Newman-Keuls multiple comparison test. The significance level was set at $p \leq 0.05$.

3. Results

3.1 Behavioral analyses

3.1.1 Novel tank test

In the novel tank test (Figure 1), two-way ANOVA of distance traveled (Figure 1A) showed a significant effect of SE diet x PQ treatment interaction ($F_{1,36}$ = 7,138, p = 0,0113). The multiple comparison test showed that PQ treatment per se caused a decrease in distance traveled when compared to CT group, while this effect was prevented in SE+PQ group. Concerning the absolute turn angle (data not shown), no significant differences were observed. The analysis of freezing episodes (Figure 1B) showed significant effects of SE diet x PQ treatment interaction ($F_{1,44}$ = 6.568, p = 0.0139) and PQ treatment ($F_{1,44}$ = 4,766, p = 0.0344). Similarly, two-way ANOVA of duration of freezing bouts (Figure 1C) showed significant effects of SE diet x PQ treatment interaction ($F_{1,44}$ = 4.441, p = 0.0408) and PQ treatment ($F_{1,44}$ = 4.553, p = 0.0385). The multiple comparison test showed that PQ treatment

per se increased both bouts and duration of freezing when compared to the other groups. SE diet prevented these effects in SE+PQ group. Two-way ANOVA of top entries (data not shown) did not demonstrate significant differences among groups, but a significant effect of PQ treatment ($F_{1,44}$ = 6.500, p = 0.0144) and SE diet ($F_{1,44}$ = 6.733, p = 0.0128) was observed regarding the time spent in top area (Figure 1D). PQ treatment increased the time spent in top when compared to CT group, and SE diet was able in prevented this effect in SE+PQ group.

3.3.2 Preference to conspecifics

In the preference to conspecifics (Figure 2), a two-way ANOVA of transitions to A1 (Figure 2A), closest to mirror area, did not demonstrate significant differences among groups. When the time spent in A1 (Figure 2B) was evaluated, the analyses showed a significant effect of SE diet ($F_{1,44}$= 25.79, p < 0.0001). PQ treatment per se did not change the time in A1 when compared to CT group. SE diet per se and SE+PQ groups spent more time in A1 when compared to CT. Considering the latency to enter in A1 segment (Figure 2C), a significant PQ treatment x SE diet interaction ($F_{1,40}$ = 11.44, p = 0.0018) and PQ treatment ($F_{1,40}$ = 7.369, p = 0.0086) was verified. PQ treatment per se increased the latency to enter in A1, while SE diet prevents this effect in SE+PQ group.

3.1.3 Mirror-induced aggression (MIA) test

In the MIA test (Figure 3), the analysis of transitions to A1 (Figure 3A), closest to mirror area, a two-way ANOVA reveled a significant effect of PQ treatment ($F_{1,48}$ = 11.16, p = 0.0016) and SE diet ($F_{1,48}$ = 26.00, p < 0.0001). All experimental groups presented fewer transitions to A1 in comparison to CT. Considering the time spent in A1 (Figure 3B), significant effects of SE diet x PQ treatment interaction ($F_{1,44}$ = 4.426, p = 0.0412) and SE diet ($F_{1,44}$ = 11.21, p = 0.0017) were observed. PQ treatment increased the time spent in A1, while this effect was prevented in SE+PQ group. A two-way ANOVA showed significant effects of SE diet x PQ treatment interaction ($F_{1,44}$ = 20.77, p<0.0001), PQ treatment ($F_{1,44}$ = 5.207, p = 0.0274) and SE diet ($F_{1,44}$ = 14.96, p = 0.0004) in the number of aggressive episodes (Figure 3C). Similarly, the duration of aggressive display (Figure 3D) also demonstrated significant effects of SE diet x PQ treatment interaction ($F_{1,44}$ = 15.27, p = 0.0003), PQ treatment ($F_{1,44}$ = 6.765, p = 0.0126) and SE diet ($F_{1,44}$ =13.75, p=0.0006). Post hoc analysis showed that PQ increased the number and duration of aggressive episodes, while pretreatment with SE prevented these effects. The analysis of the latency to attack the mirror

(Figure 3E) revealed a significant effect of SE diet ($F_{1,44}$ = 10.55, p = 0.0022), in which both SE and PQ+SE groups showed increased values in comparison to CT and PQ groups.

3.2 Biochemical analyses

3.2.1 Analyses of zMAO activity and TH expression

A two-way ANOVA of TH expression (Figure 4A) did not reveal significant effects among groups. On the other hand, a two-way ANOVA of zMAO activity (Figure 4B) revealed a significant effect of SE diet ($F_{1,16}$ = 24.64, p = 0.0003). PQ treatment per se did not show significant differences when compared with CT group. The SE diet per se and SE+PQ group increased MAOz activity. Although no significant effects were verified, pretreatment with SE presented a tendency to increase the zMAO activity.

3.2.2 Biomarkers of oxidative and nitrosative damage

A two-way ANOVA of TBARS levels (Figure 5A) revealed a significant effect of SE diet x PQ treatment interaction ($F_{1,20}$ = 18.83; p = 0.0003) and PQ treatment ($F_{1,20}$ = 5.304; p = 0.0321). All groups presented a significant decrease in TBARS levels when compared to CT group. SE diet did not cause significant effects in TBARS formation. The analysis of carbonylated protein levels (Figure 5B) revealed a significant effect of SE diet x PQ treatment interaction ($F_{1,20}$ = 16.44, p = 0.0006) and PQ treatment ($F_{1,20}$ = 16.42, p < 0.0001). PQ treatment per se induced an increase in carbonyl levels when compared to others groups, while this effect was prevented in SE+PQ group. In relation to ROS levels (Figure 5C), the two-way ANOVA revealed a significant effect of SE diet x PQ treatment interaction ($F_{1,16}$ = 5.765, p = 0.0289). PQ treatment per se increased ROS levels when compared to others groups. Pretreatment with SE diet prevented the ROS formation induced by PQ treatment. A two-way ANOVA of NOx levels (Figure 5D) revealed a significant effect of SE diet x PQ treatment interaction ($F_{1,16}$ = 17.82, p = 0.0006) and SE diet ($F_{1,16}$ = 14.54, p = 0.0015. SE diet prevented the increase in NOx levels yield by PQ.

3.2.4 Antioxidant parameters

A two-way ANOVA of SOD activity (Figure 6A) revealed a significant effect of SE diet x PQ treatment interaction ($F_{1,20}$ = 6.425, p = 0.0197), PQ treatment ($F_{1,20}$ = 21.09, p = 0.0002), and SE diet ($F_{1,20}$ = 9.274, p = 0.0064). PQ treatment and SE diet per se did not show significant differences when compared to CT group. SE+PQ group showed an increase

in SOD activity when compared to others groups. The analysis of CAT activity (Figure 6B) showed a significant effect of SE diet x PQ treatment interaction ($F_{1,20}$ = 24.00, p < 0.0001), PQ treatment ($F_{1,20}$ = 14.24, p = 0.0012), and SE diet ($F_{1,20}$ = 15.89, p = 0.0007). PQ treatment per se demonstrated a significant increase in CAT activity when compared with the other groups. SE diet prevented increase in CAT activity observed in PQ group. In relation to GPx activity (Figure 6C), two-way ANOVA yielded a significant effect of SE diet x PQ treatment interaction ($F_{1,20}$ = 88.55, p < 0.0001), PQ treatment ($F_{1,20}$ = 21.20, p = 0.0002), and SE diet ($F_{1,20}$ = 24.55, p < 0.0001). PQ treatment per se increased GPx activity when compared to other groups. Pretreatment with SE prevented the PQ-induced increase in GPx activity. In addition, SE diet per se also increased GPx activity as compared to CT and PQ groups. The analysis of GR activity (Figure 6D) showed a significant effect of SE diet x PQ treatment interaction ($F_{1,20}$ = 81.91, p < 0.0001), PQ treatment ($F_{1,20}$ = 167.8, p < 0.0001), and SE diet ($F_{1,20}$ = 94.47, p < 0,0001). PQ treatment per se showed a decrease in GR activity when compared to CT group. SE diet per se induced a significant increase in GR activity in comparison to the other groups. Pretreatment with SE was not efficient in preventing the decrease of GR activity induced by PQ treatment. A two-way ANOVA of GST activity (Figure 6E) yielded a significant effect of PQ treatment ($F_{1,20}$ = 88.68; p < 0.000) and SE diet ($F_{1,20}$ = 11.72; p = 0.0027). PQ treatment and SE diet per se increased GST activity compared with CT group. In SE+PQ group the SE pretratement did not prevent the effects caused by PQ treatment in GST activity. In NPSH levels (Figure 6F) the analysis revealed significant effects of SE diet x PQ treatment interaction ($F_{1,20}$ = 20.41, p = 0.0002) and SE diet ($F_{1,20}$ = 20.06, p < 0.0001). PQ treatment per se decreased the NPSH levels when compared to CT group. SE diet per se did not change the NPSH levels when compared to CT group, but SE diet was able in prevent the decrease in NPSH levels observed in PQ-treated fish.

3.2.5 Overview of Na_2SeO_3 action on the deleterious effects induced by PQ

The Figure 7 shows a possible mechanism by which the Na_2SeO_3 can protect the brain of the PQ-induced neurotoxicity in zebrafish. We found a protective effect of Na_2SeO_3 on behavioral and biochemical parameters. The overall result of this study reflects the real possibility of Na_2SeO_3 as a promising candidate for neuroprotective use against PQ toxicity.

3. Discussion

In the current study, we evaluated the Na_2SeO_3 as a potential neuroprotective agent against biochemical and behavioral changes induced by PQ in zebrafish. For the first time, we demonstrate that dietary Na_2SeO_3 prevents motor and non-motor alterations triggered by chronic PQ treatment in zebrafish, as well as modulates redox parameters in brain tissue of this species.

The administration of PQ in different experimental models has been associated with behavioral changes and neurochemical impairments that closely resemble those observed in PD (Jimenez-Del-Rio et al. 2010; McCormack et al. 2006; Nunes et al. 2016; Szabó et al. 1991). Previous reports showed that PQ administration in zebrafish leads to locomotors impairments and significant changes in non-motor parameters, increasing anxiety-like behaviors and aggression (Bortolotto et al. 2014; Nunes et al. 2016). Among the behavioral repertoire of zebrafish, the species gradually explore the upper portions of a novel tank, it has a have a tendency to approach to conspecifics, and displays agonistic behaviors in stressful situations (Kalueff et al. 2013).

We observed that dietary Na_2SeO_3 attenuated locomotors deficit (Figure 7A), as well as the increase in time spent in top area (Figure 7C) induced by PQ. In addition, Na_2SeO_3 diet abolished the exacerbation of freezing behavioral (Figure 7B), attenuated the increase of latency to enter the area close to conspecifics (Figure 7D), and prevented the agonistic behaviors as anxiety- and aggressive-like phenotypes (Figure 7E) detected in PQ-treated animals. Se homeostasis in the brain is important in regulating behavioral functions in zebrafish, such as swimming, stress, anxiety, and predator avoidance in fishes (Benner et al. 2010). In rats, several studies have shown that the increase of Se supply in diet may improve motor performance (Ellwanger et al. 2015; Shahar et al. 2010) and may contribute to stabilize mood disorders, cognitive impairment, anxiety, depression, and hostility (Benton et al. 2002; Cardoso et al. 2014; Ibrahim et al. 2014; Mlyniec et al. 2015; Solovyev 2015). We hypothesized that Na_2SeO_3 effects on these parameters also could be related with hormonal changes as thyroid hormone regulation, which is associated with neuropsychiatric manifestation (Khörle et al. 2010; Rayman 2000).

Tyrosine hydroxylase is the main enzyme involved in dopamine synthesis, whereas monoaminoxidase is an enzyme involved in its degradation (Meiser et al. 2013). Although PQ acts on dopamine metabolism, in our experiment, the PQ treatment did not affect the zMAO activity (Figure 7F) and TH levels. Animals exposed to Na_2SeO_3 diet per se and cotreated

with Na_2SeO_3 and PQ showed increased zMAO activity. The exact mechanism associated with the modulatory action of Se on MAOz activity is still controversial. Some authors showed that both organic and inorganic Se supplementation decrease MAO-B enzyme activity in brain of adult rats (Sampaio et al. 2016; Tang et al. 2008). Studies performed by Bortolotto et al. (2014) and Bretaud et al. (2004) also did not find differences on TH levels in zebrafish chronically exposed to PQ. As zebrafish is a relatively recent experimental model in neuroscience research, its use to mimic parkinsonism-like signals induced PQ is few explored yet. Furthermore, the mechanisms underlying PQ action on dopaminergic pathways in zebrafish are still conflicting and require further investigation.

The brain is more susceptible to oxidative stress than most other tissues due to its high oxygen consume and lower antioxidant activity (Haliwell et al. 1992; Steinbrenner and Sies 2009). Oxidative stress occurs when ROS increase in relation to antioxidant defense system and lead to oxidative damage in biomolecules, such as lipids, proteins and nucleic acids. ROS can also react with nitric oxide (NO) forming the reactive nitrogen species (RNS), which are equally deleterious. Both species are generally detoxified by cellular enzymatic and non-enzymatic antioxidant compounds, maintaining the natural redox state of the cell (Cadenas et al. 2000; Pisoschi and Pop 2015). Excessive production and/or insufficient degradation of ROS can cause oxidative damage of astrocytes and/or neurons and as consequence acute brain injury and neurodegenerative diseases (Behl and Moosman 2002).

Considering that PQ pro-oxidant effects are mainly attributed to its redox cycle, which triggers to ROS generation and subsequent oxidative and nitrosative damage, we evaluated the effects of PQ on these parameters (Figure 7G). Na_2SeO_3 pretreatment prevented the increase of CP, ROS and NOx levels caused by PQ treatment. In the context, some studies have demonstrated that Na_2SeO_3 supplementation can ameliorate the oxidative effects triggered by PQ (Blanco-Ayala et al. 2014; Kim et al. 2012). Se antioxidant activity is directly related with scavenging and reducing of ROS and RNS levels (Li et al. 2014; Steinbenner and Sies 2009) and these could be possible mechanisms by which Se acts to prevent the protein damage.

In addition, selenoproteins could be involved in neuroprotection controlling the redox status, the activation of antioxidant pathways and de novo selenoprotein synthesis (Cardoso et al. 2015; Pitts et al. 2014). Se insufficiency has been associated with enhanced brain susceptibility to oxidative damage and an increased risk of neurodegenerative diseases (Chen and Berry, 2003; Fang et al. 2013; Sharma and Amin 2013). In a mice model, Jesse et al. (2010) demonstrated that Se compounds could prevent nitrosative damage in CNS caused by

high levels of cerebral nitrate/nitrite. Moreover, there is evidence showing that Se supplementation prevents enhanced protein oxidation in brain of rats and fish exposed to toxins (Menezes et al. 2014; Monteiro et al. 2009; Moskovitz and Stadtman 2003). Thus, our study reinforces the hypothesis that Se exhibited neuroprotection due its antioxidant and antinitrosative properties, reducing oxidative/nitrosative damage.

Furthermore, previous studies showed that Se supplementation lower than 3 mg/kg had positive effects on animal growth, on feed conversion rate and did not produce adverse effects in fish (Banni et al. 2011; Hamilton 2003). Importantly, we did not observe apparent signs of Se toxicity, including abnormal swimming behavior or mortality, suggesting that the Na_2SeO_3 concentration used did not cause adverse effects in zebrafish.

Since oxidative damage triggered by PQ is basically mediated by free radicals, we investigated the status of endogenous antioxidant parameters (Figure 7H), which are the first line of defense against free radical damage. The level of thiols is an essential factor in controlling ROS generation and glutathione is the most important antioxidant molecule that acts as a ROS scavenger, neutralizing $^{\bullet}O_2^-$, $^{\bullet}OH$ and $^{\bullet}NO$. Then, H_2O_2 generated is reduced to H_2O by GPx and CAT enzymes (Pisoschi and Pop 2015; Roman et al. 2013). Our results showed that pretreatment with Na_2SeO_3 prevented the increase of CAT and GPx activities and the decrease of NPSH levels induced by PQ treatment. Se interaction with the GPx system represents the major defense system against oxidative stress in the brain (Pillai et al. 2014; Dringen et al. (2000). Cheng et al. (1999) demonstrated that mice fed with Se presented higher GPx activity and sustained less oxidative damage after PQ injection than mice fed without Se. It has been shown that SOD protects against PQ toxicity in fish (Ken et al. 2003). In our study, PQ per se unchanged SOD activity. However, Na_2SeO_3 diet in PQ-treated group induced a significant increase in SOD activity, supporting the idea that Se could prevent PQ toxicity by modulating enzymatic antioxidant defenses. Furthermore, the significant increase in GPx, CAT and GST activities suggest a compensatory mechanism in response to oxidant effects triggered by PQ, which are reported in the literature (Cochemé and Murphy 2007; Nunes et al. 2016).

Na_2SeO_3 diet yield a significant increase in GST activity per se and in PQ-treated group. Pretreatment with dietary Na_2SeO_3 was not able to maintain the enzyme levels similar to control. PQ treatment and Na_2SeO_3 diet decreased TBARS levels when compared to control, suggesting a putative relationship with the activation of GST, a detoxifying enzyme involved in the elimination of lipid peroxides. The involvement of GPx activity is also plausible since it protects tissues of H_2O_2 and lipidperoxides (Sunde et al. 2013). Despite GR

does not act directly in the removal of radical species, it is responsible for glutathione regeneration in presence of NADPH, aiming to prevent the stoppage of metabolic glutathione cycle. In our study, Se diet per se increases GR activity, but this treatment was not effective in preventing the decreased in GR activity yield by PQ exposure. The mechanism involved in PQ toxicity involves NADPH oxidation (Suntres 2002) and, considering that GR is a NADPH-dependent enzyme, the reduction in GR activity could be related with a depletion of NADPH levels in PQ-treated animals leading to a consequent impairment in recycling oxidized glutathione (GSSG) to reduced glutathione (GSH) and oxidative stress.

To our knowledge, the present study highlights for the first time that dietary Na_2SeO_3 can play a protective role on behavioral and biochemical functions impaired by PQ in zebrafish. Se diet modulates not only redox parameters, but also anxiety- and aggressive-like phenotypes. These results could be attributed, at least in part, due to its effects on non-motor parameters and also due to its antioxidant properties. Additionally, we demonstrate that zebrafish is a convenient model organism for screening potential protective molecules against the oxidative damage involved in PQ-induced neurotoxicity. Importantly, we emphasize that a more complete evaluation regarding the neural mechanisms triggered by Se in zebrafish is needed in order to clarify its protective role in fish species.

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Figure captions

Figure 1 – Novel tank test. Effects of pretreatment with Na_2SeO_3 in zebrafish PQ-treated on motor parameters as (A) distance traveled, and nonmotors parameters as (B) freezing boats, (C) during of freezing , and (D) time spent in top . Data are reported as mean ± SEM and analyzed by two-way ANOVA. Different letters indicate differences between groups ($p < 0.05$).

Figure 2 – Preference to conspecifics test. Effects of pretreatment with Na_2SeO_3 in zebrafish PQ-treated on (A) transitions to A1, (B) time spent in A1, and (C) latency to enter in A1. Data are reported as mean ± SEM and analyzed by two-way ANOVA. Different letters indicate differences between groups ($p < 0.05$).

Figure 3 - Aggression test. Effects of pretreatment with Na_2SeO_3 in zebrafish PQ-treated on (A) transitions to A1, (B) time spent in A1, (C) number of aggressive episodes in A1, (D) during of aggressive episodes in A1, and (E) latency to attack the mirror in A1. Data are reported as mean ± SEM and analyzed by two-way ANOVA. Different letters indicate differences between groups ($p < 0.05$).

Figure 4 – Analyses of zMAO activity and TH expression. Effects of pretreatment with Na_2SeO_3 on (A) zMAO activity and (B) TH expression in zebrafish PQ-treated. Data are reported as mean ± SEM and analyzed by two-way ANOVA. Different letters indicate differences between groups ($p < 0.05$).

Figure 5 - Biomarkers of oxidative and nitrosative damage. Effects of pretreatment with Na_2SeO_3 on (A) TBARS, (B) CP (C) ROS and (D) NOx levels in zebrafish PQ-treated. Data are reported as mean ± SEM and analyzed by two-way ANOVA. Different letters indicate differences between groups ($p < 0.05$).

Figure 6 – Antioxidant parameters. Effects of pretreatment with Na_2SeO_3 on (A) SOD, (B) CAT, (C) GPx, (D) GR, (E) GST activities and (F) NPSH levels in zebrafish PQ-treated. Data are reported as mean ± SEM and analyzed by two-way ANOVA. Different letters indicate differences between groups ($p < 0.05$).

Figure 7 – Overview of Na_2SeO_3 (SE) action on the deleterious effects induced by PQ on behavioral and biochemical parameters. (A,B,C) Novel tank test, (D) Preference to conspecifics test, (E) Agression test, (F) zMAO activity and TH expression, (G) Biomarkers of Oxidative and Nitrosative Damage (H) Antioxidant Parameters.

Figure 1 – Novel tank test

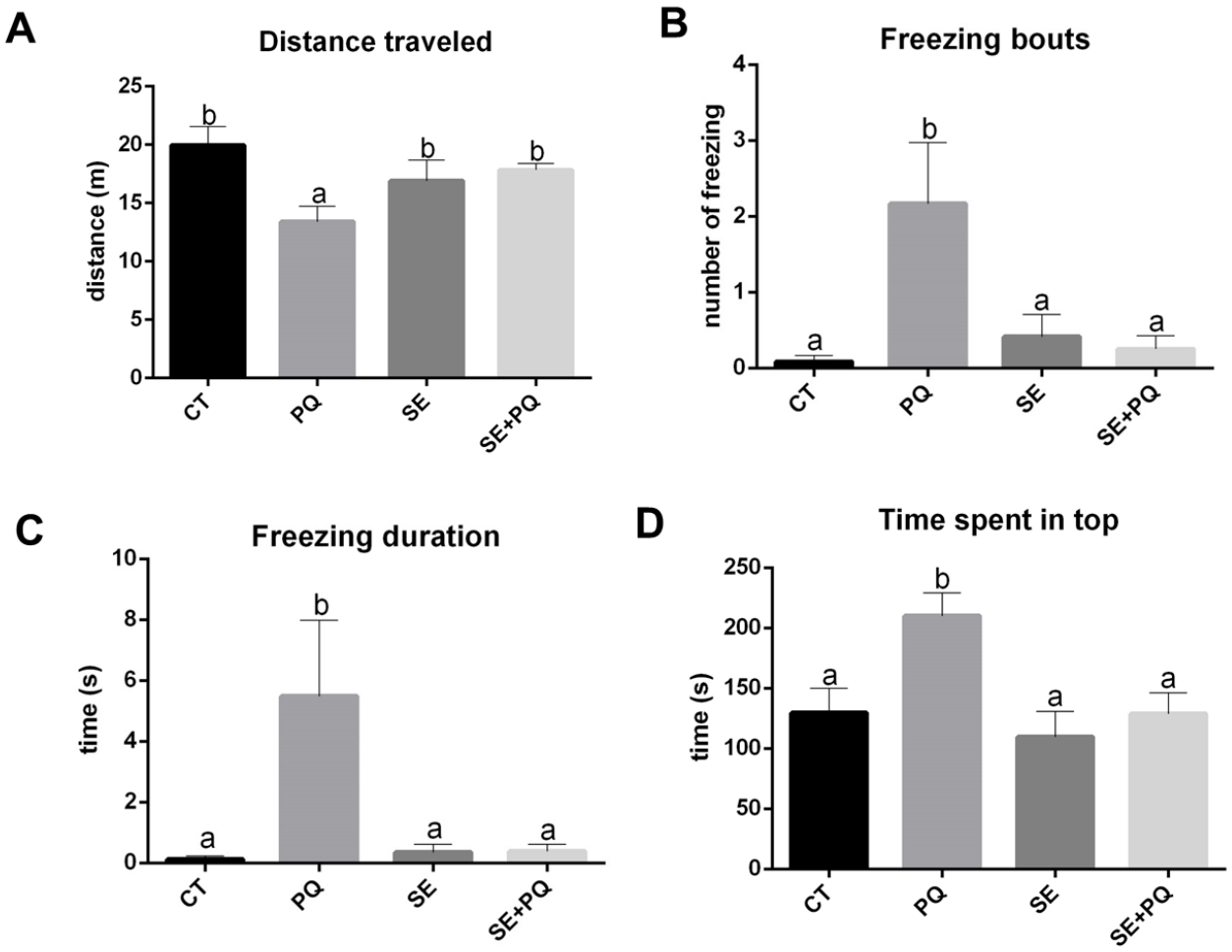


Figure 2 – Preference to conspecifics test

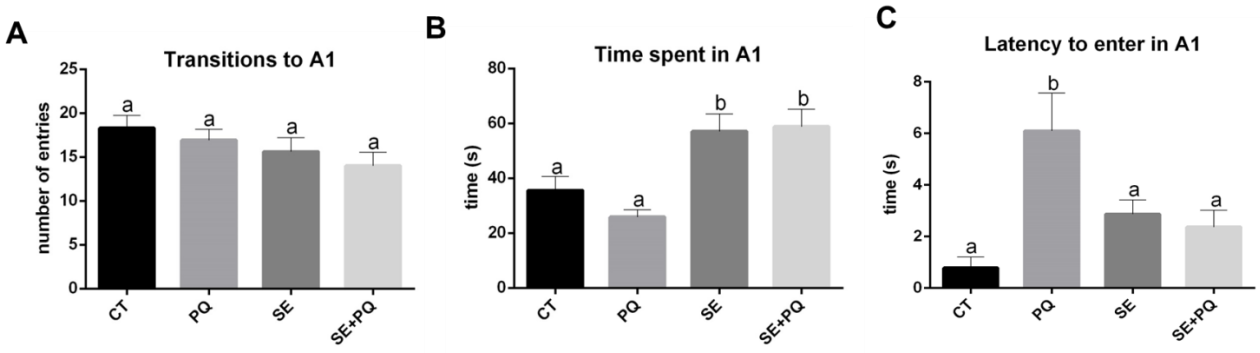


Figure 3 – Aggression test

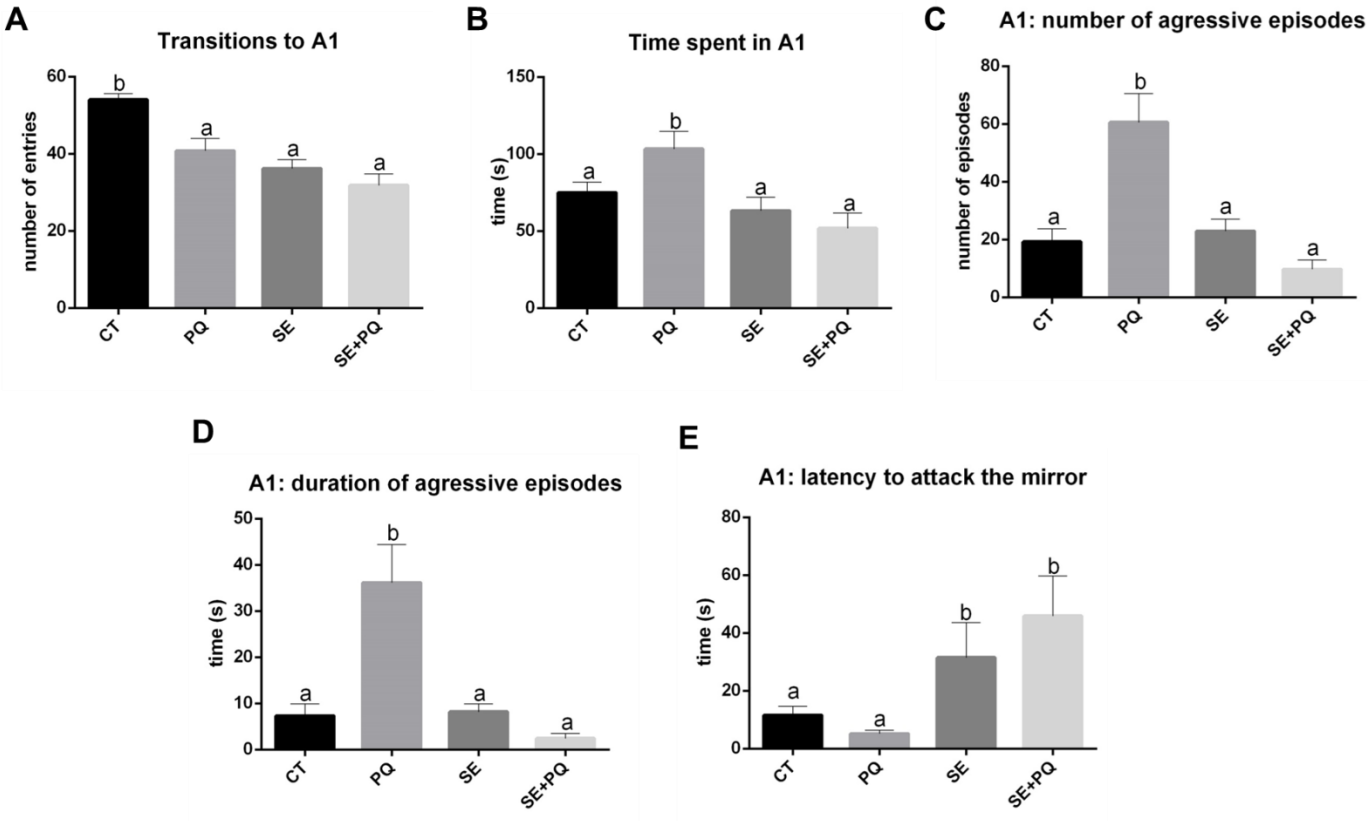


Figure 4 – Analyses of zMAO activity and TH expression

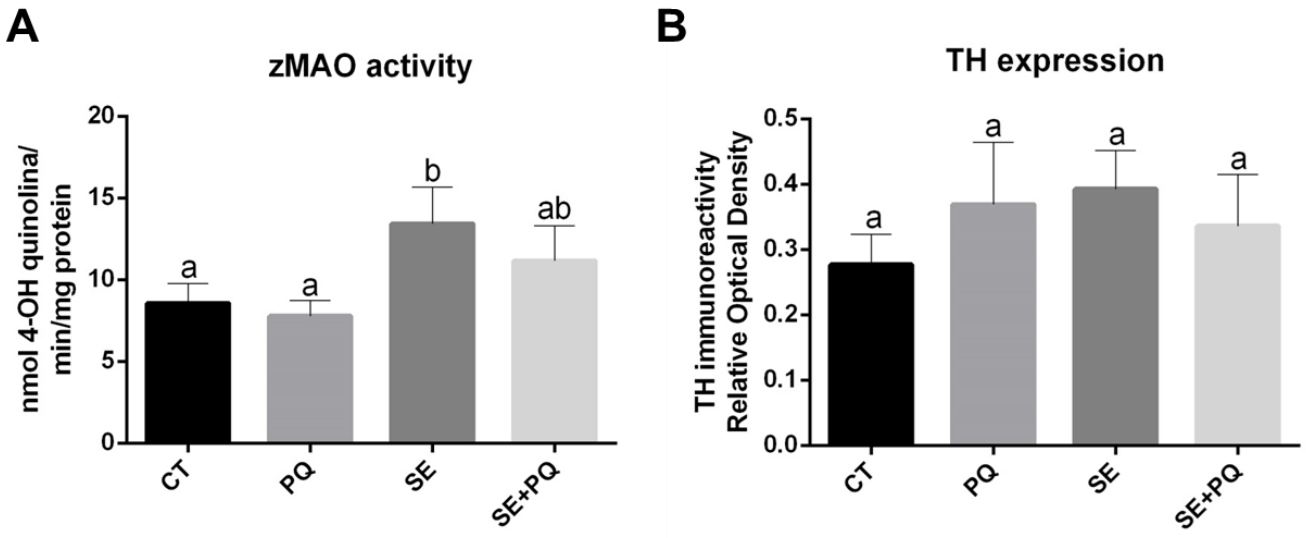


Figure 5 – Markers of oxidative and nitrosative damage

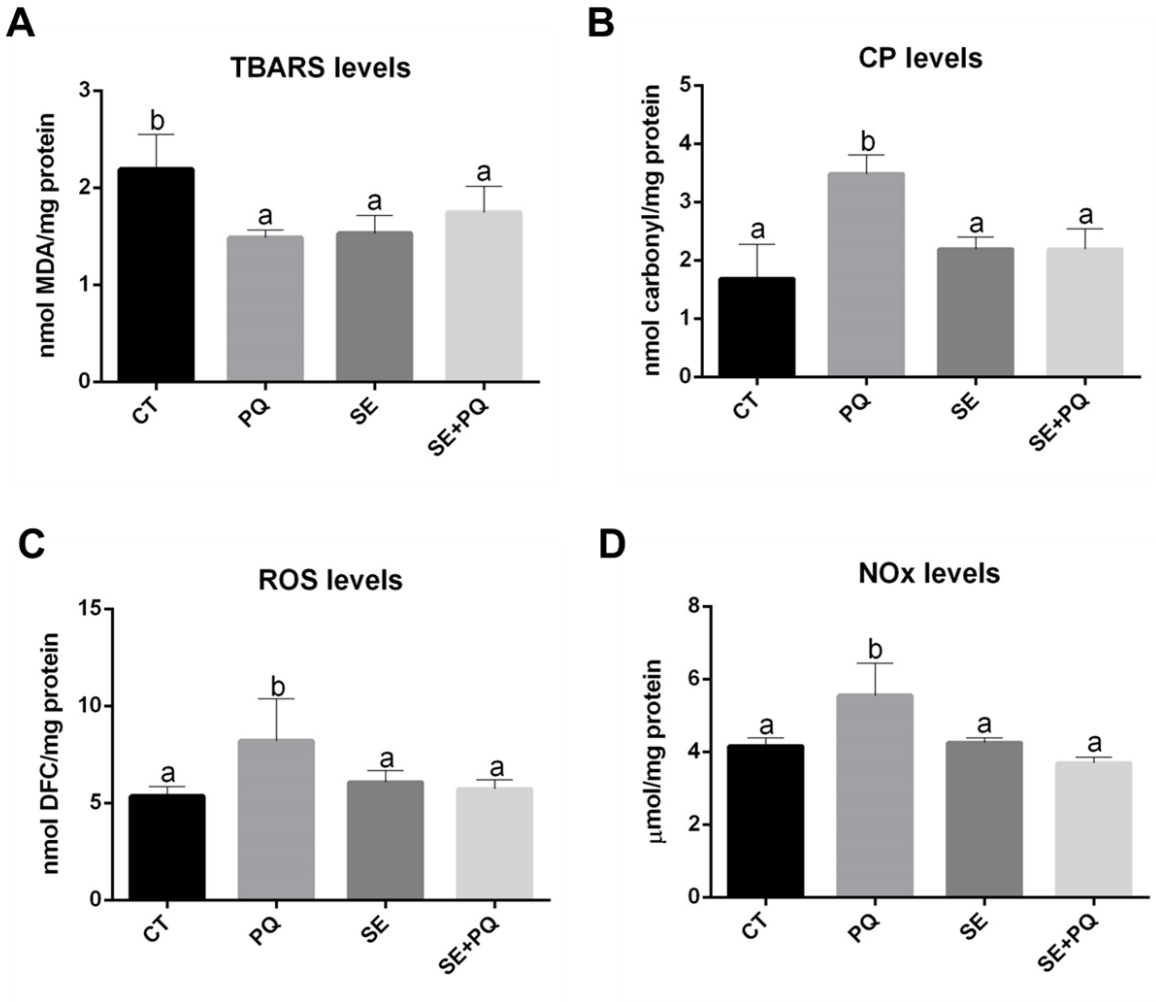


Figure 6 – Antioxidant parameters

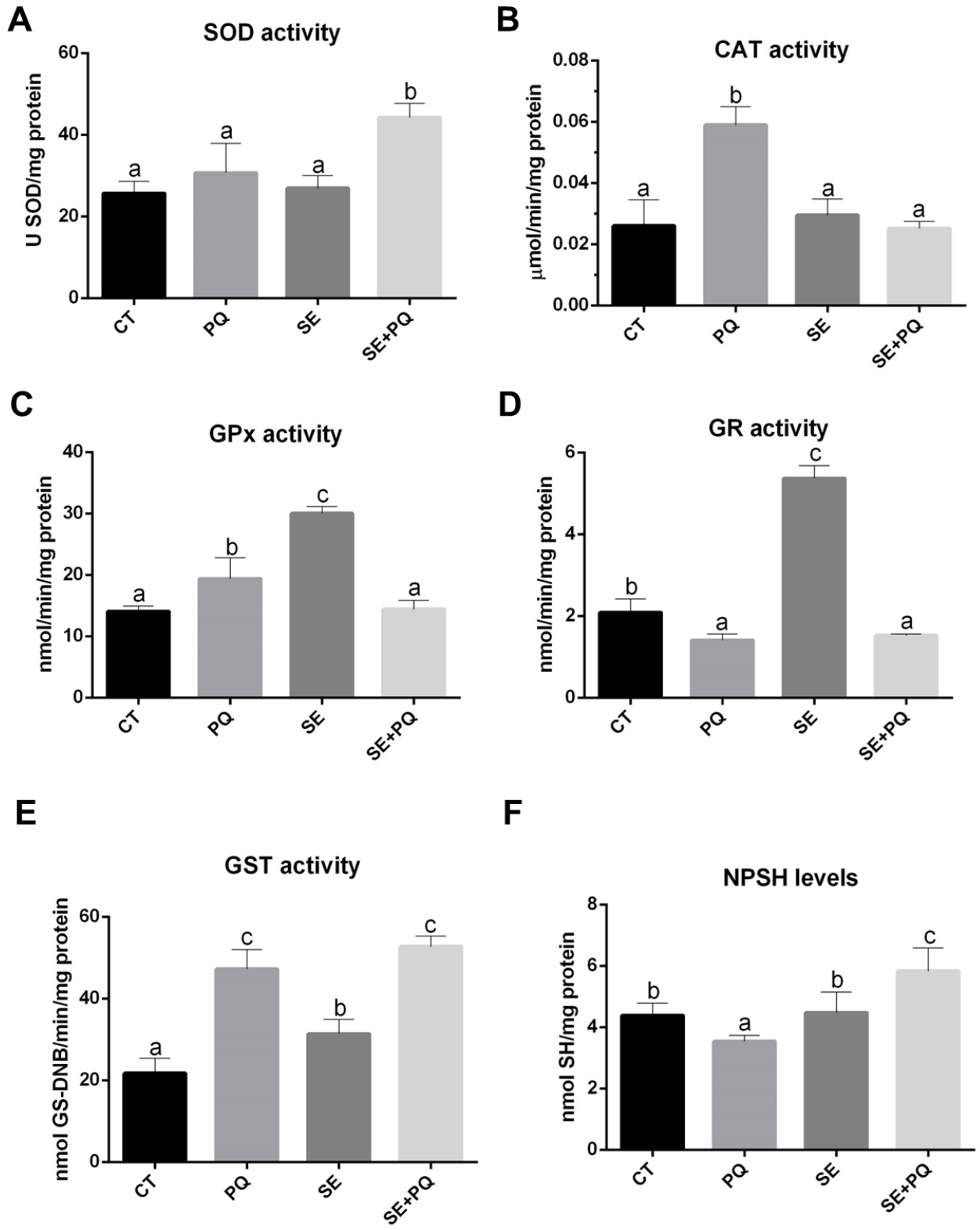
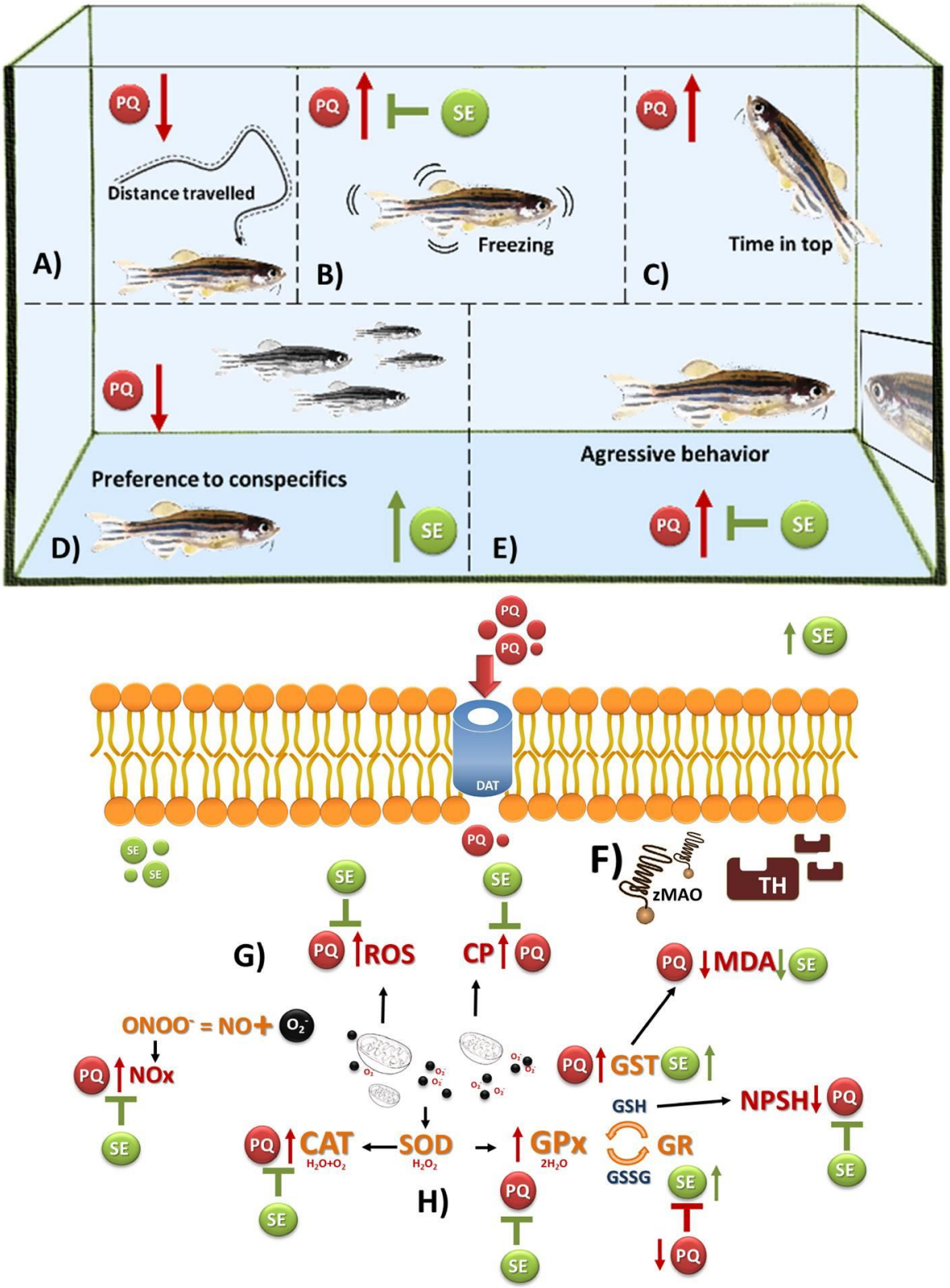


Figure 7- Overview of Na_2SeO_3 action on the deleterious effects induced by PQ



5. CONCLUSÃO

Em suma, a presente dissertação demonstra que a pré-alimentação com Na_2SeO_3 preveniu alterações comportamentais e bioquímicas em um modelo de neurotoxicidade induzido por PQ em peixe-zebra. Essa conclusão pode ser sustentava pelos seguintes dados:

- O pré-tratamento com Na_2SeO_3 foi capaz de atenuar alterações motoras induzidas pelo tratamento com PQ;

- O pré-tratamento com Na_2SeO_3 modulou parâmetros do tipo ansiedade detectados em animais tratados com PQ;

- O pre-tratamento com Na_2SeO_3 preveniu danos oxidativos e nitrosativos, como o aumento na carbonilação de proteínas, níveis de ROS e NOx induzidos pelo tratamento com PQ.

- O pre-tratamento com Na_2SeO_3 preveniu alterações no sistema antioxidante, como o aumento das enzimas CAT e GPx e a diminuição dos níveis de NPSH induzidos pelo tratamento com PQ;

- O peixe-zebra se mostrou um bom modelo experimental para o *screening* de potenciais moléculas antioxidantes contra a neurotoxicidade induzida pelo herbicida PQ.

6. PERSPECTIVAS DO ESTUDO

Este trabalho demonstrou que o Na_2SeO_3 foi um bom composto antioxidante contra a neurotoxicidade induzida pelo PQ. Dessa maneira, as perspectivas desse estudo são:

- Avaliar o potencial efeito neuroprotetor de outros compostos antioxidantes frente a este modelo experimental;

- Elucidar características relacionadas à via dopaminérgica neste modelo de neurotoxicidade induzido pelo herbicida PQ;

- Investigar "*in vitro*" o mecanismo pelo qual o Na_2SeO_3 desencadeia seu efeito neuroprotetor frente à toxicidade induzida pelo PQ.

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ANEXO A – CARTA DE APROVAÇÃO DO CEUA

Universidade Federal de Santa Maria
1960

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

CERTIFICADO

Certificamos que o Projeto intitulado "Efeitos de dieta suplementada com selênio sobre parâmetros comportamentais e bioquímicos em modelo de Parkinsonismo induzido pelo herbicida Paraquat em zebrafish (Danio rerio)", protocolado sob o CEUA nº 1777051115, sob a responsabilidade de **Vania Lucia Loro** *e equipe; Talise Ellwanger Müller; Charlene Cavalheiro de Menezes; Luciana Joner Guerra; Mauro Eugênio Medina Nunes; Tiago da Luz Fiuza; Vanessa Andreatta de Quadros* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei 11.794, de 8 de outubro de 2008, com o Decreto 6.899, de 15 de julho de 2009, com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovado** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) em reunião de 10/03/2016.

We certify that the proposal "Diet supplemented with selenium effects on behavioral and biochemical parameters in a model of Parkinsonism induced by the herbicide Paraquat in zebrafish (Danio rerio)", utilizing 300 Fishes (males and females), protocol number CEUA 1777051115, under the responsibility of **Vania Lucia Loro** *and team; Talise Ellwanger Müller; Charlene Cavalheiro de Menezes; Luciana Joner Guerra; Mauro Eugênio Medina Nunes; Tiago da Luz Fiuza; Vanessa Andreatta de Quadros* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes (or teaching) - it's in accordance with Law 11.794, of October 8 2008, Decree 6899, of July 15, 2009, with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 03/10/2016.

Vigência da Proposta: de 01/2016 a 03/2017 Área: Bioquímica E Biologia Molecular

Procedência:	Biotério externo						
Espécie:	Peixes	sexo:	Machos e Fêmeas	idade:	2 a 2 meses	N:	300
Linhagem:	Danio rerio			Peso:	0,4 a 0,6 g		

Resumo: A doença de Parkinson (DP) é uma doença neurodegenerativa que afeta boa parte da população idosa no mundo. Os mecanismos pelos quais a doença é adquirida ainda não são bem esclarecidos, o que dificulta a busca por compostos que atuem na prevenção ou na reversão desse quadro. O mineral selênio é um antioxidante exógeno de grande relevância e diversos estudos demonstram sua contribuição no tratamento e prevenção de doenças através do combate ao estresse oxidativo. Porém, a atuação do selênio na DP ainda é pouco conhecida e sua propriedade antioxidante pode estar sendo pouco explorada para a prevenção e tratamento da DP. O objetivo do nosso estudo é investigar o potencial efeito protetor do selênio na forma de Selenito de Sódio, administrado pela dieta, em parâmetros bioquímicos e comportamentais em um modelo de Parkinsonismo induzido por Paraquat em zebrafish (Danio rerio).

Santa Maria, 11 de março de 2016

Profa. Dra. Daniela Bitencourt Rosa Leal
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Prof. Dr. Denis Broock Rosemberg
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria