UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA

Tiago da Luz Fiuza

EFEITOS DA DIETA SUPLEMENTADA COM DISSELENETO DE DIFENILA SOBRE A TOXICIDADE INDUZIDA POR MERCÚRIO EM CAMUNDONGOS E PEIXES

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

Orientador: Prof. Dra. Vania Lucia Loro

Co-orientador: Prof. Dra. Maria Ester Pereira

Santa Maria, RS

2019

Fiuza, Tiago da Luz
EFEITOS DA DIETA SUPLEMENTADA COM DISSELENETO DE DIFENILA SOBRE A TOXICIDADE INDUZIDA POR MERCÚRIO EM CAMUNDONGOS E PEIXES / Tiago da Luz Fiuza.- 2019.
84 p.; 30 cm

Orientadora: Vania Lucia Loro
Coorientadora: Maria Ester Pereira
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, RS, 2019

1. Disseleneto de Difenila 2. Mercúrio 3. Nefrotoxicidade 4. Camundongos 5. Peixes I. Loro, Vania Lucia II. Pereira, Maria Ester III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

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Aprovado em 18 de janeiro de 2019:

Vania Lucia Loro, Dra. (UFSM)
Presidente/Orientador

Nilda Berenice Barbosa, Dra. (UFSM)

Bernardo Baldisserotto, Dr. (UFSM)

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Claudia Bueno dos Reis Martinez, Dra. (UEL) –
Vídeo conferência

Santa Maria, RS
2019

AGRADECIMENTOS

Agradeço primeiramente à professora Vania Lucia Loro, pessoa que me recebeu tão bem em seu grupo e tornou possível a realização deste trabalho. Agradeço pelo credito que me foi confiado, pelos ensinamentos, amizade e paciência. Também à professora Maria Ester Pereira, que como co-orientadora deste trabalho sempre dispôs do seu tempo para lançar a ajuda necessária e aconselhamentos. Serei eternamente grato por ter me concedido a oportunidade de ser seu aluno de mestrado, mostrando sempre através do seu exemplo o caminho digno e correto. Muito obrigado.

Agradeço a meus pais e irmãos pela força, incentivo e inspiração. Muito obrigado.

Agradeço aos colegas de trabalho, Luciana Joner Guerra, Jossiele Leitemperger, Eduardo Severo, Aline Blank e Aline Marins por toda ajuda e aconselhamento no decorrer deste trabalho. Obrigado por tornar a realização deste trabalho possível. Aos demais colegas dos laboratórios LabTAq, MetaboLab e LaNE: Dionatan, Graciele, Leticia, Maiara, Taisson, Jamile Bernardi e em especial (de coração) Vanessa Andreatta por toda a ajuda prestada, companheirismo e amizade.

A Universidade Federal de Santa Maria e ao Programa de Pós-Graduação Ciências Biológicas: Bioquímica Toxicológica pela possibilidade de realização deste trabalho. Aos demais professores, funcionários e colegas do Programa de Pós-Graduação Ciências Biológicas: Bioquímica Toxicológica que de alguma maneira contribuíram para minha formação.

À CAPES pela Bolsa de Pós-Graduação concedida.

OBRIGADO!

RESUMO

EFEITOS DA DIETA SUPLEMENTADA COM DISSELENETO DE DIFENILA SOBRE A TOXICIDADE INDUZIDA POR MERCÚRIO EM CAMUNDONGOS E PEIXES

AUTORA: TIAGO DA LUZ FIUZA
ORIENTADOR: VANIA LUCIA LORO

Com o aumento de atividades industriais e agrícolas, diversos contaminantes são liberados no meio ambiente. Contaminantes como o mercúrio (Hg) podem ser provenientes do uso das práticas agrícolas, queima de combustíveis fósseis e atividades de mineração, sendo que esta atividade se destaca no Brasil. Uma vez liberado no meio ambiente o Hg atinge os ambientes aquáticos e terrestres e consequentemente os organismos que ali habitam. Com o objetivo de minimizar possíveis efeitos tóxicos em organismos vivos, o uso de aditivos alimentares com propriedades antioxidantes pode representar uma boa alternativa. O disseleneto de difenila $(PhSe)_2$ é um composto orgânico de selênio com propriedades antioxidantes, e possui efeitos benéficos na terapêutica de animais expostos a metais pesados. Dessa forma, o objetivo deste trabalho foi avaliar o efeito de uma dieta suplementada com $(PhSe)_2$ sobre a toxicidade induzida pelo cloreto de mercúrio ($HgCl_2$). Jundiás adultos (40 - 50g) e camundongos *Swiss* albinos (25–30g) foram tratados durante 30 dias consecutivos com ração suplementada com $(PhSe)_2$ ou ração controle. Após 25 dias de tratamento os animais receberam uma dose diária de solução de $HgCl_2$ (1,7mg/kg, peixes, 5,0mg/kg camundongos) durante 5 dias. Os animais foram eutanasiados 24h após a última administração de $HgCl_2$ e os tecidos sanguíneo, renal e hepático foram coletados para análise da atividade da enzima δ-aminolevulinato desidratase (δ-ALA-D). Foram avaliados parâmetros de toxicidade hepática (aspartato aminotransferase AST e alanina aminotrasferase ALT), renal (uréia e creatinina) e parâmetros oxidativos (TBARS, tióis totais e não proteicos e espécies reativas de oxigênio). Os peixes expostos ao Hg apresentaram inibição da atividade da δ-ALA-D do rim e sangue, aumento nos níveis de creatinina sérica e diminuição dos níveis de TBARS, tióis totais e tióis não protéicos de rim. O Hg acumulou-se no fígado e nos rins dos peixes causando alteração na homeostase do zinco (Zn) nos tecidos hepático e sanguíneo. A alimentação com $(PhSe)_2$ preveniu parcialmente a inibição da δ-ALA-D renal, elevação dos níveis de creatinina sérica, diminuição dos níveis de TBARS e tióis não proteicos, bem como a alteração na homeostase do Zn no sangue. Os camundongos expostos ao Hg apresentaram inibição da atividade da δ-ALA-D sanguínea e ALT sérica, aumento nos níveis de ureia e creatinina, diminuição dos níveis de TBARS e tióis total renal e aumento nos níveis de tióis não proteicos no rim. O Hg acumulou-se no fígado e no rim dos camundongos e causou alteração na homeostase do Zn nos rins. O $(PhSe)_2$ preveniu totalmente a inibição da δ-ALA-D de sangue, o aumento na ureia e creatinina e a diminuição dos tiois totais de rim e parcialmente a inibição da ALT sérica. Considerando os resultados, acredita-se que o $(PhSe)_2$ pode vir a ser um aditivo em potencial contra a intoxicação por Hg tanto para peixes como para mamíferos.

Palavras-chave: Disseleneto de Difenila. Mercúrio. δ-Aminolevulinato Desidratase. Estresse Oxidativo.

ABSTRACT

EFFECTS OF DIET SUPPLEMENTED WITH DIPHENYL DISSELLENE ON MERCURY-INDUCED TOXICITY IN MICE AND FISH

AUTHOR: TIAGO DA LUZ FIUZA
ADVISOR: VANIA LUCIA LORO

With the increase of industrial and agricultural activities, several contaminants are released into the environment. Contaminants such as mercury (Hg) can come from the use of agricultural practices, burning of fossil fuels and mining activities, and this is highlighted in Brazil. Once released into the environment Hg reaches the aquatic and terrestrial environments and consequently the organisms that live there. In order to minimize possible toxic effects on non-target organisms, the use of food additives with antioxidant properties may be a good alternative. Diphenyl diselenide $(PhSe)_2$, is an organic compound of selenium with antioxidant properties, and has beneficial effects in the treatment of animals exposed to heavy metals. Thus, the objective of this work was to evaluate the effect of a diet supplemented with $(PhSe)_2$ on mercury chloride ($HgCl_2$) -induced toxicity. Adult jundias (40-50g) and albino Swiss mice (25-30g) were used which were treated for 30 consecutive days with feed supplemented with $(PhSe)_2$ or control ration. After 25 days of treatment the animals received a daily dose of $HgCl_2$ solution (1.7mg / kg, fish, 5.0mg / kg mice) for 5 days. The animals were euthanized 24 hours after the last administration of HgCl2 and blood, renal and hepatic tissues were collected for analysis of the activity of the enzyme δ-aminolevulinate dehydratase (δ-ALA-D). Hepatic toxicity parameters (aspartate aminotransferase AST and alanine aminotransferase ALT), renal (urea and creatinine) and oxidative parameters (TBARS, total and non-protein thiols and reactive oxygen species) were evaluated. Fish exposed to Hg showed inhibition of δ-ALA-D activity of kidney and blood, increase in serum creatinine levels and decrease of TBARS, total thiols and non-protein thiols of kidney. Hg accumulated in the liver and kidneys of the fish causing alteration in zinc (Zn) homeostasis in the liver and blood tissues. Feeding with $(PhSe)_2$ partially prevented the inhibition of renal δ-ALA-D, elevated serum creatinine levels, decreased levels of TBARS and non-protein thiols, as well as alteration in blood Zn homeostasis. Mice exposed to Hg showed inhibition of blood δ-ALA-D and serum ALT activity, increased urea and creatinine levels, decreased levels of TBARS and total renal thiols and increased levels of non-protein thiols in the kidney. Hg accumulated in the liver and kidney of mice and caused alteration in kidney Zn homeostasis. O $(PhSe)_2$ completely prevented inhibition of blood δ-ALA-D, increase in urea and creatinine, and decrease of total kidney thiols and partially inhibition of serum ALT. Considering the results, it is believed that $(PhSe)_2$ may prove to be a potential additive against Hg poisoning for both fish and mammals.

Keywords: Diphenyl diselenide. Mercury. δ-Aminolevulinate Dehydratase. Oxidative stress

LISTA DE ABREVIATURAS

ALT: alanina aminotransferase

AST: aspartato aminotransferase

ANOVA: análise de variância (analysis of variance)

°C: grau Celsius

DNA: ácido desoxirribonucléico

δ-ALA-D: delta-aminolevulinato desidratase

EO: estresse oxidativo

ERO: espécies reativas de oxigênio

GPx: glutationa peroxidase

Gr: glutationa redutase

GSH: glutationa reduzida

h: hora

Hg: mercúrio

Hg°: mercúrio metálico; mercúrio elementar

m: mili

M: molar

μ: micro

min: minuto

n: número de repetições

NPSH: tióis não protéicos

p: nível de significância

PBG: porfobilinogênio

pH: potencial hidrogeniônico

rpm: rotações por minuto

s.c.: subcutânea; subcutâneamente

S.E.M.: standard error of mean (erro padrão da média)

SH: grupos sulfidrílicos

SOD: superóxido dismutase

TCA: ácido tricloroacético

TSH: tióis totais

U: unidades

v/v: volume/volume

Zn: zinco

SUMÁRIO

1. APRESENTAÇÃO

Esta Tese aborda assuntos relacionados aos efeitos mediados de uma dieta suplementada com $(PhSe)_2$ sobre a toxicidade induzida pelo cloreto de mercúrio ($HgCl_2$) em parâmetros toxicidade, utilizando jundiás adultos (*Rhamdia quelen*) e camundongos *Swiss* albinos como organismos modelos. Ela encontra-se estruturada da seguinte forma:

INTRODUÇÃO: Revisão da literatura com caracterização dos temas abordados na tese.

RESULTADOS: Serão apresentados na forma de artigo científico e manuscrito.

DISCUSSÃO: serão apresentados as interpretações e comentários gerais sobre o artigo e o manuscrito científico.

CONCLUSÃO: Comentários gerais sobre os resultados obtidos no trabalho.

REFERÊNCIAS: Lista as referências utilizadas na introdução e discussão da Tese.

2. INTRODUÇÃO

2.1 Contaminação ambiental

O aumento das atividades industriais e agrícolas, bem como o crescimento populacional observado nas últimas décadas, desencadeia a liberação de uma série de contaminantes e poluentes no meio ambiente, principalmente no ecossistema aquático (HANSDA; KUMAR; USMANI, 2014; SROGI, 2008). Além disso, áreas com intensa atividade industrial contribuem para a liberação de substâncias tóxicas, incluindo metais pesados, cuja ação afeta os organismos em seu habitat natural (GBEM et al., 2001; WOODLING; BRINKMAN; HORN, 2001). Os metais são um dos muitos tipos de contaminantes encontrados no meio ambiente, dentre eles, podemos citar o cádmio, chumbo e o mercúrio, conhecidos por exercer efeitos tóxicos uma vez que possuem facilidade em acumular-se no organismo (PRATUSH; KUMAR; HU, 2018). As ações de origem antropogênicas são responsáveis por adições de até 1,16 milhões de toneladas de metais por ano em ecossistemas terrestres e aquáticos no mundo (STREETS et al., 2017). No Brasil, a mineração de níquel, ouro, ferro e de outros metais de interesse comercial tem contribuído com a liberação de rejeitos, constituindo uma das principais formas de contaminação do solo e da água por metais pesados (GUILHERME et al., 2005).

A utilização industrial de produtos contendo esses elementos tem gerado preocupações a respeito dos principais efeitos adversos causados ao meio ambiente, bem como para a saúde humana. Estudos têm demonstrado que a exposição de animais a esses compostos pode induzir danos em diversos tecidos, bem como alterações no metabolismo (FIUZA et al., 2015; MORAES-SILVA et al., 2012; OLIVEIRA et al., 2014, 2015). O acúmulo de metais nos ecossistemas aquáticos tem despertado grande interesse em pesquisas cientificas cujo objetivo é investigar os efeitos desses contaminantes nas cadeias alimentares, no comportamento de animais, e na ciclagem biogeoquímica (LA COLLA; BOTTÉ; MARCOVECCHIO, 2019). A cadeia de contaminação de metais quase sempre segue a ordem cíclica, partindo da indústria seguindo na atmosfera, solo, água, fitoplâncton, zooplâncton, peixes e finalizando nos seres humanos (KADAR; KONCZ; FEKETE, 2000). A absorção de metais em peixes pode ocorrer através da superfície do corpo e através das brânquias (via respiração e/ou troca iônica com a água), bem como pelo trato digestivo (HEATH, 1995). Além disso, é importante ressaltar que de acordo com órgãos de saúde, 90% da ingestão de metais pesados ocorre por meio da

ingestão de alimentos, como por exemplo, consumo de peixes expostos a esses metais (VIRGA; GERALDO; SANTOS, 2007).

2.2 Mercúrio

O mercúrio (Hg) é um metal divalente pertencente à família 2B, ou família do Zinco (Zn), da tabela periódica. Este metal não essencial, ou seja, sem função biológica, encontra-se no estado líquido mesmo em temperatura ambiente. O Hg pode ser encontrado em duas formas químicas: inorgânica (Hg^0 – elementar; Hg^{2+} - sais de mercúrio) e orgânica (MeHg – metilmercúrio), sendo que as amálgamas dentárias, água de beber e o consumo de peixes são as principais formas de exposição a este metal. Todas as formas de Hg são tóxicas para os diferentes organismos vivos, contudo, os efeitos tóxicos, distribuição e toxicocinética dependem da forma química, nível e tempo em que o organismo foi exposto. Por exemplo, o cloreto de mercúrio ($HgCl_2$) é nefrotóxico, enquanto o metil mercúrio é hepatotóxico e neurotóxico (CLARKSON, 1997; COUNTER; BUCHANAN, 2004; FARINA; ASCHNER; ROCHA, 2011; GRAEME; POLLACK, 1998; WHO, 2003).

Como já descrito anteriormente, regiões com intensa atividade industrial e mineração tornam-se foco de liberação de Hg para o meio ambiente, dessa forma o Hg torna-se um poluente ambiental através dessas ações de origem antropogênica. Entretanto, a liberação de Hg para o meio ambiente também provêm de regiões que apresentam intensas atividade vulcânicas e geotermais, porém estas caracterizam as formas naturais de liberação desse metal para a biosfera (LI et al., 2009; WHO, 1991). Na atmosfera, o Hg metálico é oxidado pelo ozônio, formando o cloreto de mercúrio. Este sal de mercúrio inorgânico deposita-se na água e no solo, onde pode ser metilado por bactérias anaeróbicas, formando metilmercúrio, ou se volatilizar, retornando ao ambiente (BISINOTI; JARDIM, 2004). As emissões de origem antropogênica representam 70% de todo o Hg liberado no meio ambiente e contribuem para o aumento da contaminação por Hg em escala local, regional e global. No entanto, diversas agências nacionais e internacionais já apontam este metal para um possível controle de emissões. Este agente está presente em combustíveis energéticos e em lixos municipais, sendo um dos metais que apresentam menor eficácia de retenção em processos de controle de emissão (AZEVEDO; NASCIMENTO; CHASIN, 2001; PIRRONE et al., 2010, 2010).

No Brasil, o principal foco de contaminação ocorre na Amazônia, onde a prática do garimpo é intensa, sendo que o Hg é jogado nos rios para amalgamar o ouro durante o

processo de extração, seguido pelo uso na agricultura e indústria (BERZAS NEVADO et al., 2010; PALHETA; TAYLOR, 1995). Populações ribeirinhas são alvos potenciais do mercúrio, pois habitam locais onde as águas e fauna aquática estão contaminadas pelos rejeitos derivados da atividade de extração de ouro. A baixa renda dessas populações agrava a exposição ao Hg, uma vez que a principal fonte alimentar proteica provém do consumo de peixes originários do ambiente contaminado (CASTILHOS et al., 2006; CHAN et al., 2003; LEBEL et al., 1996; RENZONI; ZINO; FRANCHI, 1998). Além disso, o incidente ocorrido no município de Mariana – MG, onde uma barragem de rejeitos da produção de minério de ferro rompeu, controlada pela Samarco Mineração S.A, lançou cerca de 60 milhões de metros cúbicos de rejeitos em forma de lama, ocasionando a devastação da região e contaminando o Rio Doce, principal recurso hídrico do estado de MG. O Serviço Autônomo de Água e Esgoto (SAAE) de Baixo Guandu – ES, em laudos preliminares, confirmou a presença de metais como chumbo, mercúrio, arsênio e ferro nas águas do Rio Doce (LACAZ; PORTO; PINHEIRO, 2016). A bacia hidrográfica do Rio Doce abrange 230 municípios dos estados de Minas Gerais e Espírito Santo e a lama que chegou ao rio impactou muitas dessas populações, que eram abastecidas por suas águas.

2.3 Mercúrio Inorgânico

Cloreto de mercúrio ($HgCl_2$) é a principal forma inorgânica do mercúrio, possui o estado de oxidação Hg^{2+} e é altamente hidrofílico, o que faz com que seja nessa forma que o Hg desencadeie sérios danos ao sistema renal. Além disso, a eliminação urinaria é a principal via de eliminação de metais tóxicos, o que faz com que os rins estejam constantemente expostos a agentes tóxicos (AU, 2004; FOWLER, 1993). A ligação deste metal com grupamentos SH de proteínas, peptídeos e aminoácidos leva à necrose do epitélio renal, acompanhado de proteinúria e glomerulonefrite (GOYER R.; KLAASSEN; WALLKES M.P, 1995). Este metal também promove estresse oxidativo (EO), peroxidação lipídica e disfunção mitocondrial (ZALUPS, 2000). Estudos realizados em nosso laboratório já demonstraram que o $HgCl_2$ pode induzir o aumento nos níveis de ureia e creatinina, bem como alterações no perfil oxidativo, o que evidencia danos ao tecido renal (DA LUZ FIUZA et al., 2018; FIUZA et al., 2015; MESQUITA et al., 2016; OLIVEIRA et al., 2015).

A forma inorgânica tem sido alvo de inúmeras pesquisas cientificas, além de sua toxicidade intrínseca é atribuída à ela a toxicidade do mercúrio elementar e, parcialmente, a

do metilmercúrio, uma vez que, por oxidação e desmetilação, respectivamente, ambas as formas são convertidas em Hg^{2+} (COUNTER; BUCHANAN, 2004). Portanto, mesmo que o sistema renal seja o alvo principal do Hg inorgânico, o tecido hepático também é prejudicado por esta forma do metal (BERLIN; ZALUPS; FOWLER, 2007). A hepatotoxicidade dessa forma de mercúrio ainda não está totalmente elucidada, no entanto, assim como nos rins, acredita-se que ocorra a interação do metal com estruturas orgânicas, permitindo ao metal transpor a membrana dos hepatócitos mais facilmente (BERLIN; ZALUPS; FOWLER, 2007; ROONEY, 2007).

A toxicidade do $HgCl_2$ pode ser atribuída a sua alta afinidade com grupamentos sulfidrílicos (-SH), contudo, esse metal pode causar uma mudança estrutural em enzimas e por consequência dificultar a ligação de grupos prostéticos, ou até mesmo, bloquear os sítios ativos dessas enzimas (GUNNAR et al., 2007). A δ-aminolevulinato desidratase (δ-ALA-D) é um exemplo de enzima que possui grupamentos -SH em seu sítio ativo e é geralmente utilizada como um marcador de exposição a metais divalentes (BERNARD; LAUWERYS, 1987). Estudos realizados em nosso laboratório demonstraram que ratos expostos ao $HgCl_2$ apresentaram uma diminuição na atividade da enzima δ-ALA-D em tecidos como rim, fígado e cérebro (DA LUZ FIUZA et al., 2018; FIUZA et al., 2015; MESQUITA et al., 2016; MORAES-SILVA et al., 2012; OLIVEIRA et al., 2014, 2015).

Estudos sugerem que um importante mecanismo envolvido no dano renal induzido pela forma inorgânica do mercúrio envolve a indução do estresse oxidativo (AGARWAL et al., 2010). A alta afinidade do mercúrio com grupamentos SH nos componentes celulares induz a diminuição de outros grupamentos SH (especialmente GSH) do interior da célula, dessa forma, aumentando a predisposição das células tubulares proximais ao estresse oxidativo (FUKINO et al., 1984). Agarwal e colaboradores (2010) demonstraram que ratos expostos ao Hg apresentaram uma diminuição na atividade das enzimas de defesa antioxidante: superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GPx) e glutationa redutase (GR), em tecido hepático, renal e cerebral.

2.4 Selênio

O selênio (Se) é um elemento químico que pertence à família 16 da tabela periódica (calcogênios) sido descoberto em 1817 pelo químico sueco Jöns Jacob Berzelius. Esse elemento químico apresenta-se em 4 estados de oxidação: Selenato (Se+6), selenito (Se+4), selênio elementar (Se0) e seleneto (Se-2) (STADTMAN, 1980). Diferentemente do mercúrio

o selênio é um elemento traço essencial para os sistemas biológicos de mamíferos e de peixes. Possui ação antioxidante devido ao fato de fazer parte do sítio ativo de diversas enzimas. Dentre essas enzimas, podemos citar a glutationa peroxidase e tioredoxina redutase, que atuam protegendo as membranas celulares dos danos causados pelo processo de oxidação (HAMILTON, 2004). Considerando a importância deste micronutriente, a suplementação de dietas com selênio, tanto para animais quanto para humanos, tem sido aceita pela comunidade científica. Para humanos, a Junta de Alimentação e Nutrição da Academia de Ciências dos Estados Unidos propõe uma ingestão diária de 50-200 mg de selênio, a qual é considerada segura e saudável para adultos. Este elemento pode ser encontrado em alimentos como castanha-do-pará, alho, cebola, brócolis, cogumelos, cereais, pescados, ovos e carnes (DUMONT; VANHAECKE; CORNELIS, 2006).

Considerando a importância do Se como um micronutriente essencial para o organismo, principalmente como antioxidante, seu uso como agente terapêutico tem despertado o interesse de pesquisadores (PAPP et al., 2007). Contudo, sabe-se que o Se possui efeito na distribuição do Hg no organismo, podendo reduzir a toxicidade causada por este metal (GOYER R.; KLAASSEN; WALLKES M.P, 1995). Assim, não apenas o Se na sua forma elementar, mas também os compostos orgânicos de selênio tem despertado grande interesse, tanto na sua importância como intermediários em síntese orgânica, bem como por apresentarem propriedades farmacológicas em diferentes modelos experimentais (BARBOSA et al., 2008; NOGUEIRA; ROCHA, 2010; NOGUEIRA; ZENI; ROCHA, 2004).

Inicialmente os organocalcogênios foram alvos de interesse para os químicos devido as suas aplicações como intermediários para a síntese orgânica (PETRAGNANI; RODRIGUES; COMASSETO, 1976). Posteriormente as pesquisas se voltaram para a busca de organocalcogênios com atividade biológica, visando sua utilização como compostos com atividades farmacológicas, destacando, dessa forma, os compostos orgânicos de selênio (NOGUEIRA; ZENI; ROCHA, 2004; PARNHAM; GRAF, 1991). Diversos estudos demostraram a capacidade de compostos orgânicos ou inorgânicos de Se em reverter a toxicidade causada pelo Hg. No entanto, os compostos orgânicos possuem menor toxicidade quando comparados aos inorgânicos (FARINA et al., 2003; PEROTTONI et al., 2004; SASAKURA; SUZUKI, 1998).

2.5 Disseleneto de difenila $(PhSe)_2$

O disseleneto de difenila $(PhSe)_2$ é um composto orgânico de Se lipofílico. Possui propriedades farmacológicas, cuja ação antioxidante se destaca, uma vez que o $(PhSe)_2$ possui atividade semelhante a enzima glutationa peroxidase (NOGUEIRA; ZENI; ROCHA, 2004). Além disso, estudos em animais de laboratório indicam que o $(PhSe)_2$ possui atividade protetora contra danos induzidos por diferentes xenobióticos, incluído o Hg e outros metais pesados. Contudo, estudos demonstraram que o $(PhSe)_2$ apresentou atividade protetora contra o estresse oxidativo induzido pelo herbicida Quinclorac em peixes (CAVALHEIRO DE MENEZES et al., 2012). Em adição, o $(PhSe)_2$ também demostrou ser eficaz em reverter os danos oxidativo induzidos pelo cádmio em fígado, rim, sangue e cérebro de camundongos (SANTOS et al., 2004). Além disso, dados da literatura demonstram que o pré-tratamento com $(PhSe)_2$ foi efetivo em proteger contra as alterações hematológicas causadas pelo $HgCl_2$ (BRANDÃO et al., 2008). Experimentos ex vivo demonstraram que o $(PhSe)_2$ protege contra os danos renais induzidos pelo cloreto de mercúrio tanto em peixes como em mamíferos. Essa proteção foi confirmada pela redução dos níveis de ureia e creatinina, além de alterar a distribuição do Hg entre os tecidos dos animais que receberam $(PhSe)_2$ e foram expostos ao Hg, sugerindo um efeito benéfico deste organocalcogênio no tecido renal (BRANDÃO et al., 2006; DA LUZ FIUZA et al., 2018; FIUZA et al., 2015). Entre outras atividades farmacológicas que o $(PhSe)_2$ possui, podemos citar a ação antidepressiva e ansiolítica (SAVEGNAGO et al., 2008), anti-inflamatória (NOGUEIRA; ZENI; ROCHA, 2004), antiúlcera (SAVEGNAGO et al., 2006), efeito neuroprotetor (GHISLENI et al., 2003), hepato-protetora (BORGES et al., 2005, 2006), anti-hiperglicêmica (BARBOSA et al., 2006) e possível retardo no desenvolvimento de câncer (BARBOSA et al., 2008). Contudo, poucos estudos demonstrando os benefícios de uma alimentação suplementada com $(PhSe)_2$ contra os efeitos tóxicos do Hg são encontrados na literatura, principalmente em peixes.

2.6 A utilização de modelos animais na pesquisa

Os peixes possuem um importante papel na cadeia alimentar, portanto avaliar os efeitos tóxicos e a acumulação Hg em peixes é importante para a avaliação de efeitos adversos destes contaminantes principalmente para a saúde humana. O jundiá (*Rhamdia quelen*) é uma espécie nativa da América do Sul e Central e tem despertado grande interesse no sul do Brasil devido suas características, tais como resistência ao manejo, crescimento rápido, boa eficiência alimentar e carne saborosa (BARCELLOS; KREUTZ; QUEVEDO, 2004; CARNEIRO; MIKOS, 2005). Além disso, o jundiá apresenta boa aceitação pelo mercado

consumidor, tanto para a pesca quanto para a alimentação, sendo uma espécie com excelentes características para o processamento (CARNEIRO, 2004).

Fonte: https://www.planetcatfish.com/common/species.php?species_id=872 (Acessado e adaptado em janeiro de 2019).

Além disso, pesquisas com o camundongo *Swiss* albino também são importantes, uma vez que possuem alto grau de similaridade com os seres humanos (±85%), podendo ser útil para elucidar os efeitos contaminantes por metais na ação comportamental e bioquímica em mamíferos. Essa espécie de animal de laboratório tem sua origem no continente Asiático e é a mais utilizada em pesquisas cientificas na área biomédica. Dentre suas vantagens em pesquisas laboratoriais podemos citar o seu pequeno tamanho, alta proliferação, período de gestação curto, fácil domesticação e manutenção. Por essas características, tornou-se um dos mamíferos mais usados na experimentação mundial. Além disso, graças a sua uniformidade genética, todos os indivíduos reagem praticamente da mesma maneira a uma determinada situação experimental, ou seja, a variância dos resultados experimentais é muito pequena. Dessa forma, o pesquisador pode utilizar um número menor de animais, extremamente relevante do ponto de vista econômico e uma das principais recomendações do ponto de vista da ética no uso de animais na pesquisa (ANDRADE; PINTO; OLIVEIRA, 2002; MAGALHÃES, 2012).

Fonte: https://www.taconic.com/mouse-model/nmri (Acessado em Janeiro de 2019).

2.7 Parâmetros de Toxicidade

A ureia e a creatinina são os principais indicadores de dano agudo ao tecido renal, sendo muito utilizados na prática clínica (EDELSTEIN, 2008). A amônia, excretada pelos peixes, é resultante do catabolismo das proteínas. Nos mamíferos a amônia é convertida em ureia, que é mais hidrossolúvel e menos tóxica, sendo este resíduo o principal produto de excreção de material nitrogenado em mamíferos. A creatinina, por sua vez, é formada pelo metabolismo normal da musculatura devido à degradação da fosfocreatinina. Ambos os metabólitos são excretados pelos rins, portanto, o aumento de seus níveis no sangue indica falha na função renal (RAVEL, 1997). Alguns estudos têm relacionado a toxicidade renal causada pelo mercúrio com o aumento sanguíneo de ureia/amônia e creatinina em roedores e peixes (AGARWAL et al., 2010; DA LUZ FIUZA et al., 2018; FIUZA et al., 2015; PEIXOTO; PEREIRA, 2007).

A alanina aminotransferase (ALT) é uma enzima localizada no citosol e encontra-se em tecidos como rins e coração, porém sua localização ocorre principalmente no tecido hepático. A aspartato aminotransferase (AST) é uma enzima mitocondrial e está distribuída em diversos tecidos do organismo como o coração, músculo esquelético, rins, cérebro e pulmões, o que diminui a sua especificidade para a lesão hepática. A ALT plasmática é considerada o marcador com maior sensibilidade e relativamente mais específico para hepatotoxicidade (ANTOINE et al., 2009; OZER et al., 2008; WHITEHEAD et al., 1999).

O $HgCl_2$ altera a atividade da ALT possibilitando a utilização dessa enzima como marcador de hepatotoxicidade (VEDAVATHI; GIRISH; KUMAR, 2004). Recentemente

verificou-se uma inibição da ALT sérica de ratos jovens expostos ao $HgCl_2$, onde foi sugerido uma provável relação do metal com grupamentos sulfidrílicos presentes na estrutura da enzima (FIUZA et al., 2015; FRANCISCATO et al., 2011; MORAES-SILVA et al., 2012; PEIXOTO et al., 2007). Outro exemplo de enzima que possui grupamentos -SH em seu sítio ativo é a δ-aminolevulinato desidratase (δ-ALA-D). Esta enzima está presente na maioria dos tecidos, tem como substrato o ácido delta aminolevulínico e catalisa a condensação de duas moléculas assimétricas deste ácido para formar o composto monopirrólico porfobilinogênio (Figura 3), participando assim da rota da síntese do heme (SASSA, 1982). A inibição dessa enzima pode levar ao acúmulo do ácido δ-aminolevulínico, que possui efeito pró-oxidante, levando à geração de radicais livres e, consequentemente, aumenta o dano oxidativo nos componentes celulares (BECHARA, 1996).

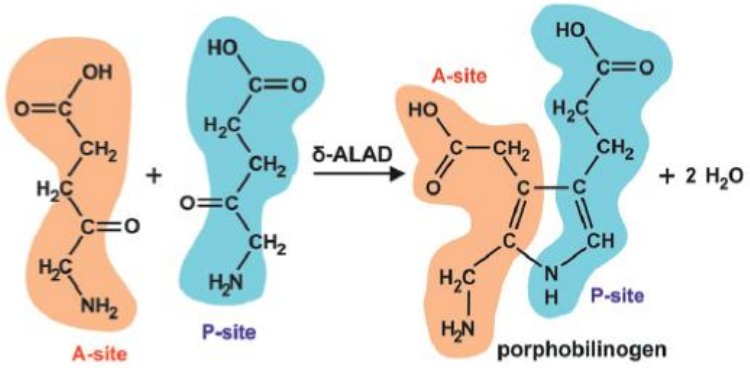

Figura 3. Síntese de porfobilinogênio (PBG) a partir de duas moléculas do ácido 5-aminolevulínico (δ-ALA) (adaptado de Rocha et al. 2012).

Em mamíferos, esta enzima requer o zinco (Zn) como co-fator no seu sítio ativo, o qual possui um único sítio de ligação do zinco com três resíduos de cisteína (GODWIN, 2001; JAFFE, 1995). Estes grupamentos sulfidrílicos estão envolvidos na coordenação essencial do íon Zn, e a proximidade entre eles torna a enzima particularmente sensível a oxidação. O íon Zn está envolvido também na estabilização destes grupos sulfidrílicos e a sua remoção pode acelerar o processo de auto-oxidação da enzima perdendo assim sua capacidade catalítica. Devido à proximidade entre os grupamentos SH presentes no sítio ativo, a δ-ALA-D torna-se bastante sensível à oxidação por metais divalentes como o Hg ou o Pb, por exemplo, pois esses metais possuem a capacidade de remover o Zn presente no sítio ativo e ligarem-se aos grupamentos sulfidrílicos, fazendo com que esta perca sua atividade catalítica (MARKHAM et al., 1993; SARAIVA et al., 2012).

Durante muito tempo a enzima δ-ALA-D foi considerada um marcador importante de exposição ao chumbo em humanos, uma vez que ele é um inibidor clássico da enzima δ-ALA-D. Contudo, a inibição desta enzima por outros metais divalentes torna-a um marcador inespecífico de toxicidade para diferentes metais. Como o Hg é um metal não essencial, a enzima δ-ALA-D pode ser utilizada com um marcador de exposição para este metal (ROELS et al., 1976; THOMPSON; JONES; BEASLEY, 1977). Dados na literatura já mostram que tanto animais jovens (PEIXOTO et al., 2007) como adultos (OLIVEIRA et al., 2014), quando expostos ao Hg apresentam uma inibição da atividade da enzima δ-ALA-D.

Portanto, conhecendo os efeitos que a contaminação com Hg desencadeia, tanto em meio ambiente aquático, quanto em solos e em diferentes espécies de animais, incluindo seres humanos, acredita-se que avaliar os efeitos protetores do $(PhSe)_2$ sob os efeitos tóxicos desse metal em diferentes espécies de animais seja importante para uma possível estratégia terapêutica contra os efeitos tóxicos provenientes da exposição ao mercúrio.

3. OBJETIVOS

3.1 Objetivo geral

Avaliar os efeitos de uma suplementação alimentar com o $(PhSe)_2$ sobre os efeitos tóxicos do $HgCl_2$, bem como sua distribuição entre os órgãos de peixes e camundongos e fazer um comparativo entre os modelos experimentais.

3.2 Objetivos específicos

Avaliar o efeito da suplementação com $(PhSe)_2$ na dieta de jundiás e camundongos sobre a toxicidade do $HgCl_2$, através:

- da atividade da (δ-ALA-D) em fígado, rins e sangue de camundongos e peixes, sendo este parâmetro utilizado como marcador de exposição ao Hg;
- da análise dos níveis séricos de ureia e creatinina, como marcadores da função renal;
- da atividade da alanina aminotranferase (ALT) e aspartato aminotransferase (AST) séricas como marcadores da função hepática;
- de parâmetros oxidativos não enzimáticos como tióis totais e não proteicos e TBARS de fígado e rim;
- da mensuração dos níveis de Hg e Zn em rins, fígado e sangue, para avaliar a distribuição desses elementos no organismo.

4. ARTIGO CIENTÍFICO

Molecular Biology Reports
https://doi.org/10.1007/s11033-018-4433-z

ORIGINAL ARTICLE

Effects of diphenyl diselenide diet on a model of mercury poisoning

Tiago da Luz Fiuza[1] · Jossiele Leitemperger[1] · Eduardo Stringini Severo[2] · Aline Teixeira Marins[2] · Aline Blank do Amaral[2] · Maria Ester Pereira[1,3] · Vania Lucia Loro[1,2,3]

Received: 28 June 2018 / Accepted: 9 October 2018

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Tiago da Luz Fiuza[a], Jossiele Leitemperger[a], Eduardo Stringini Severo[b], Aline Teixeira Marins[b], Aline Blank do Amaral[b], Maria Ester Pereira[a], and Vania Lucia Loro[a,b*]

[a] Programa de Pós-Graduação em Bioquímica Toxicológica, [b]Programa de Pós-Graduação em Biodiversidade Animal. Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

Corresponding Author:

(*) Dr. Vania Lucia Loro

Departamento de Bioquímica e Biologia Molecular

Universidade Federal de Santa Maria

97105-900 - Santa Maria, RS, Brazil

Phone: +55 55 3220-9456

E-mail: vania.loro@gmail.com

Abstract

This work investigated the preventive effect of diphenyl diselenide [$(PhSe)_2$] against the toxic effects of mercury in silver catfish (*Rhamdia quelen*). The animals were treated during thirty consecutive days with a $(PhSe)_2$ supplemented feed (3.0 mg/kg^{-1}) or commercial feed. During the last five days the animals received a daily intraperitoneal dose of $HgCl_2$ (1.7 mg/kg^{-1}) or Saline (0.9%). Twenty-four hours after the last $HgCl_2$ injection, the animals were euthanized by spinal cord section to biological material obtainment. Hepatic (AST and ALT) and renal (ammonia and creatinine) toxicity biomarkers, δ-ALA-D activity, TBARS, total and non-protein thiols levels and hepatic, renal and blood mercury (Hg) and zinc (Zn) content were evaluated. Considering renal parameters, $HgCl_2$ exposition increased serum creatinine levels and decreased δ-ALA-D activity, total and non-protein thiols and TBARS levels. $HgCl_2$ exposure also decreased blood δ-ALA-D activity. With exception of blood δ-ALA-D activity and total thiols levels, $(PhSe)_2$ supplementation partially prevented mercury induced alterations. Animals exposed to $HgCl_2$ presented an increase in liver and kidney Hg content and a decrease in liver and blood Zn content. The alteration in blood Zn content was partially prevented with $(PhSe)_2$ supplementation. With the exception of mercury and zinc content, no effects of $HgCl_2$ exposure on hepatic tissue were observed. These results show that $(PhSe)_2$ supplementation can represent a promising alternative to prevent the toxic effects presented by Hg exposure.

Keywords: **δ-ALA-D.** Fish. Nefrotoxicity. Suplemented Diet. Mercury. Selenium

Introduction

Increase of industrial and agricultural activities results in several pollutants released to the environment, mainly to the aquatic ecosystem [1]. Heavy metals are among these pollutants commonly found in the environment. Many of these heavy metals induce toxic effects and easily accumulate in the tissues. Mercury is released in the environment by natural or anthropogenic activities being the latter responsible for 70% of the mercury presented in the environment [2-3]. Mercury can be found in organic and inorganic form and both are toxic to different living organisms, whose primary target are the central nervous and renal systems, respectively [4]. The Hg toxicity can be attributed to its great affinity to sulfhydryl group; its binding to biomolecules containing this radical results in reduction of antioxidant defenses and unbalance between pro and antioxidants. Mercury also binds competitively with the body's essential elements, such as zinc and selenium, leading to their elimination, disturbing several physiological and regulatory functions [5-6]. Selenium (Se) is an essential trace element important for the antioxidant system, once it is required in the active site of the enzyme glutathione peroxidase as a cofactor for its enzymatic activity. Due to its important role in the body's antioxidant defense, there is a growing research to understand its biological role, in particular, its use as a therapeutic agent [7-8]. Several studies using trace elements such as Se and Zn were conducted and the results demonstrate that they have the ability to prevent the toxicity caused by Hg [9-10]. However, the use of fish as an animal model in this kind of study is still scarce in the literature.

Diphenyl diselenide $(PhSe)_2$ is a simple synthetic organic selenium compound, which has been considered a potential pharmacological agent in several experimental models [11-12]. This organic selenium compound reduces lipid peroxidation by increasing antioxidant defenses in fish [13]. In addition, $(PhSe)_2$ protects renal tissue from the damage induced by Hg and also alters the distribution of this metal in tissues of rodents [9].

Several studies use fish as an indicator of substances toxicity [14-15-16]. The silver catfish (*Rhamdia quelen*) is a fish species with an extensive geographic distribution, occurring from Southern Mexico to central Argentina. Its features, such as tolerance to management, omnivorous feeding behavior, ability to grow throughout the winter, and high yield, place it in a prominent position among the native species of commercial interest [17].

Therefore, considering that Hg contamination may reach aquatic environments, this work aimed to evaluate the efficacy of a diet supplemented with $(PhSe)_2$ against the damage caused by mercury chloride exposure. Hepatic, renal and blood tissues alterations as well as mercury accumulation and zinc homeostasis was evaluated using silver catfish as an experimental model.

Materials and Methods

Chemicals

Reagents were obtained from Sigma Chemical Co. (St. Louis, MO) and standard commercial suppliers. Biochemical dosages were performed with commercial kits obtained from Kovalent do Brasil Ltda (Rio de Janeiro, Brazil) and Labtest Diagnóstica S.A. (Minas Gerais, Brazil). Hg and Zn standards (99.5% purity) were purchased from Merck (Darmstadt, Germany). $(PhSe)_2$ (99.9% chemical purity) was synthesized according to the method described by Paulmier [18].

Animals

Female and male adults silver catfish (*Rhamdia quelen*) weighing 50 – 60 g were obtained from EMATER (RS, Brazil) and transferred to our experimental laboratory. They were kept

on a 12 h light/dark cycle, a 20± 2°C room temperature, fed twice a day. The control and supplemented feed [(PhSe)2] were formulated with ingredients to satisfy known nutrient requirements of fish development. The experiment was in accordance with the Committee on Care and Use of Experimental Animals from the Federal University of Santa Maria, Brazil under protocol number: (2067170815), which authorizes the use of male and female silver catfish (*Rhamdia quelen*) to the execution of the present study as well as all the biochemical tests performed.

Experimental protocol and feed preparation

Animals were initially distributed in two experimental groups: the animals were fed during thirty days with control or $(PhSe)_2$ (3.0 mg/kg^{-1}) supplemented feed. In the last five days, the animals of each group were redistributed in other two groups: treated daily with $HgCl_2$ (1.7mg/kg^{-1}) or saline (vehicle) by intra peritoneal injection (i.p.); thus resulting in four experimental groups: Control–saline (control group), $(PhSe)_2$–saline (Se group), Control–Hg (Hg group) and $(PhSe)_2$–Hg (Se–Hg group). Twenty-four hours after the last $HgCl_2$ or saline administration, animals were euthanized by spinal cord section. Blood, renal and hepatic tissues samples were collected and processed according to described in "tissue preparation".

Diets with selenium were prepared according to Menezes et al. [16] that uses previous tested concentrations of $(PhSe)_2$ for *Rhamdia quelen*. Briefly, supplemented diphenyl diselenide (3.0 mg/Kg^{-1}) was added to special vitamin mineral premix prepared without selenium. All the ingredients were mixed and pelletized adding distilled water (40%–60% of ingredient weight) before further homogenizations. Pellets of approximately 5 mm of diameter were formed by grinding the mixture through a meat grinder. Control feed was prepared with the same ingredients but without $(PhSe)_2$ addition. After forming the pellets, control and (PhSe)2 supplemented feed ration were stored at 4°C until they were used. To

maintain homogeneity of feeding process, the feed pellets were released slowly into the tank until satiety of all animals. This procedure was performed twice daily throughout the experimental period.

Tissue preparation

Tubes containing heparin anticoagulant were used for the packaging of the blood samples (1 mL approximately): one part was used for the blood δ-ALA-D activity assay (200 µL) and other (300 µL) was centrifuged at 2000 × *g* for 10 min at room temperature to obtain the plasm. Plasm was used to determine ammonia and creatinine levels, aspartate (AST) and alanine aminotransferase (ALT) activities. For the δ-ALA-D activity assay, blood samples were transferred to a recipient with distilled water, in 1:4 (v/v) proportion, under constant agitation on ice bath for 10 min to full hemolysis. Kidneys and liver were quickly removed, placed on ice and homogenized in 5 and 7 volumes, respectively, of Tris–HCl buffer (10 mM, pH 7.4) to obtain the homogenate. The homogenates were centrifuged at 8000 × g during 30 min at 4°C. The low-speed supernatants (S1) were separated and used for δ-ALA-D activity, total thiols, non-protein thiols and TBARS levels determination. Furthermore, 500 µL of blood, kidney and liver (0.1 g) were removed and frozen at −20°C until Hg and Zn level determination.

*δ-**ALA-D activity***

δ-ALA-D activity was determined across the rate of product formation (porphobilinogen, PBG). The incubation system containing 100 µM of phosphate buffer (pH 7.4) and the substrate δ-aminolevulinic acid was previously pipetted. After addition of 100 µL of hepatic S1 or 200 µL of blood hemolyzed or renal S1, the incubation was initiated and carried out for 30, 60 and 90 min for liver, blood and kidney, respectively, at 39°C. The reaction was stopped by the addition of trichloroacetic acid (TCA) 10% containing $HgCl_2$

0.05 M. The PBG formation was measured with Ehrlich's reagent (4-dimethylamino benzaldehyde), using the molar absorption coefficient of 6.1×10^4 for Ehrlich-PBG salt. The absorbance was determined spectrophotometrically at 555 nm and nmol PBG/h/mg protein was the unit used to express the specific enzymatic activity according to Sassa 1982 [19].

Serum ALT and AST activity

ALT and AST activities were determined using a commercial kit in a medium containing Tris–HCl buffer 55.8 mM pH 7.14, L-alanine 500 mM, 2-oxoglutarate 15 mM and NADH 0.18 mM, with 50 µL of serum. The absorbance was determined at 340 nm and the enzymatic activity were expressed in U/L. The technical procedures were previously described by Thomas 1998 [20].

Serum ammonia levels

Ammonia levels were determined using Labtest commercial kit. The ammonium ions react with salicylate and sodium hypochlorite under the catalytic action of sodium nitroprusside in alkaline medium to form the color compound indophenol blue. 10 µL of serum sample was added to a medium containing phosphate buffer (19.34 mM pH 6.9), sodium salicylate (58.84 mM), sodium nitroprusside (3.17 mM), sodium hydroxide (0.07 M), sodium hypochlorite (3.01 mM) and incubated for 10 min at 39ºC for color development. The absorbance was measured spectrophotometrically at 600 nm. The results were expressed in mg of ammonia per dL of serum.

Serum creatinine levels

Creatinine levels were determined using a Labtest commercial kit. The estimation of creatinine serum levels was carried by measuring the quantity of creatine formed by the creatinine aminohydrolase, and using creatinine as standard. 50 µL of serum sample was

added to a medium containing picric acid (20.2 mM) and NaOH (145.4 mM) and incubated at 37°C. The absorbance was measured spectrophotometrically at 510 nm. The results were expressed in mg of creatinine per dL of serum.

TBARS assays

The lipid peroxidation was determined according to the method described by Ohkawa et al. 1979 [21], through the measurement of the thiobarbituric acid-reactive species (TBARS). 200 μL of renal or hepatic S1 were incubated with 300 μL of thiobarbituric acid (TBA) (0.8%), 200 μL of SDS (8.1%) and 500 μL of acetic acid buffer (2.5 M, pH 3.4) for 2 h at 95°C. The absorbance was measured spectrophotometrically at 532 nm. A MDA standard curve (0, 0.15, 0.3, 0.6, 1.2 and 1.5 nmol MDA) was constructed to express the results as nmol MDA/mg protein.

Total thiol and non-protein thiol (NPSH) level determination

Liver and kidney thiol (SH) levels were determined in S1 as previously described by Ellman 1959 [22]. The protein fraction contained in S1 was precipitated using TCA 4% in 1/1 proportion, followed by centrifugation at 2000 × g during 10 min. The S2 obtained was used for analysis. The absorbance was determined at 412 nm. To calculate SH in tissue samples, a curve was constructed using glutathione as standard. The thiol levels were expressed as nmol SH/mg protein.

Hg and Zn content determination

Hg and Zn content was determinate by inductively coupled plasma atomic emission (ICPE-9000 Shimadzu Scientific Instruments). About 0.2 g of kidney and liver and 0.5 ml of blood were placed in vials and frozen at – 20°C for digestion. The digestion was performed as described in Ineu et al. 2013 [23] using distilled HNO_3. The analytical Hg and Zn standard

(Merck®) was used to construct the standard curve (0, 50, 150, 300, 600, 1200 ppb) (r = 0.99334) and (0, 10, 30, 60, 120, 240 ppb) (r = 0.99614), respectively.

Protein determination

Protein content was determined according Bradford 1976 [24], using bovine serum albumin as a standard.

Statistical analysis

Results were analyzed by one-way ANOVA followed by Duncan's multiple range test when appropriate. Results were considered significant when $p \leq 0.05$. Groups statistically equal are represented by the same letters, and groups statistically different are represented by different letters.

Results

δ-ALA-D activity

Hepatic, renal and blood δ-ALA-D activity are shown in Figure 1. The $(PhSe)_2$ supplementation and mercury chloride exposure did not cause any alteration in liver δ-ALA-D enzyme activity (Fig.1A). Mercury chloride exposure induced a δ-ALA-D activity inhibition in kidney (Fig. 1B) and blood (Fig. 1C). Supplementation with $(PhSe)_2$ partially prevented the kidney δ-ALA-D inhibition, but not blood δ-ALA-D inhibition (Duncan $p \leq 0.05$). Supplementation with $(PhSe)_2$ alone (Se Group) did not cause any significant alteration in δ-ALA-D enzyme activity.

Hepatic and renal Toxicity Parameters

Serum ALT and AST Activities, serum ammonia and creatinine levels

Hepatic toxicity biomarker ALT and AST activity and the renal toxicity biomarkers ammonia and creatinine levels are shown in Table 1. The ALT and AST activity was not affected by $(PhSe)_2$ supplementation and Hg treatment. Ammonia levels were not altered by treatment with mercury chloride or $(PhSe)_2$ supplementation. Alteration in creatinine levels was observed: Hg increased creatinine levels when compared to the control and Se groups, which was partially prevented by $(PhSe)_2$ supplementation (Duncan $p \leq 0.05$). $(PhSe)_2$ supplementation *per se* did not affect creatinine levels.

Oxidative Parameters Assays

TBARS Levels

Hepatic and renal TBARS levels are shown in Figure 2. Liver TBARS levels (Fig. 2A) was not affected by treatment with $(PhSe)_2$ and mercury chloride exposure. Mercury chloride exposure decreased TBARS levels in renal tissue (Fig. 2B). This alteration in renal TBARS levels was partially prevented by $(PhSe)_2$ supplementation (Duncan $p \leq 0.05$). No significant alterations in kidney TBARS levels was observed with $(PhSe)_2$ *per se*.

Total (TT) and non-protein thiols (NPSH) determinations

The levels of TT and NPSH in liver and kidney are shown in Figure 3 and 4, respectively. The $(PhSe)_2$ supplementation and mercury chloride exposure did not cause any alteration in TT and NPSH levels from liver (Fig. 3A and 4A, respectively). Mercury chloride exposure decreased TT and NPSH levels from kidney. $(PhSe)_2$ supplementation had no effect in kidney TT levels decrease but partially avoided the kidney NPSH levels decrease caused by mercury chloride exposure (Fig. 4B) (Duncan $p \leq 0.05$). The $(PhSe)_2$ supplementation *per se*

did not cause any significant alteration in TT and NPSH levels from kidney and liver tissues when compared to the control group.

Hg and Zn content determination

Hg Content

The Hg content in liver, kidney and blood are shown in Table 2. Liver and kidneys from animals of the two groups exposed to mercury presented an increase of Hg content. Nevertheless, no measurable Hg content was found in the blood of any animal including those that belonged to groups exposed to mercury. Supplementation with $(PhSe)_2$ did not cause any change in the distribution of Hg between tissues.

Zn Content

The Zn content in liver, kidney and blood are shown in Table 2. Mercury chloride exposure induced depletion in the hepatic and blood Zn content. Only in blood Zn content alteration was partially prevented by the $(PhSe)_2$ supplementation. However, Hg exposure and $(PhSe)_2$ supplementation did not cause any alteration in Zn content in kidney.

Discussion

Mercury contamination in environmental is a real problem around the world, thus this study was conducted in order to evaluate the effects of the diet supplemented with (PhSe)2 on silver catfish (*Rhamdia quelen*) exposed to mercury. In line with toxicity effects, the δ-ALA-D activity was inhibited in blood and kidney exposed to mercury. The effects of mercury on δ-ALA-D is probably due to its ability to bind with the sulfhydryl groups present in active site of enzyme. Mercury also displace the zinc which is necessary for its catalytic activity. Some

authors showed similar effects on δ-ALA-D considering mercury interference [25-26-27]. Santos et al. 2016 [15] injected lead (Pb) in a sub-lethal dosage in Nile tilapia and verified a blood and liver δ-ALA-D inhibition, corroborating the hypothesis that metals affect negatively this enzyme. Unlike mercury which has a predilection for the renal tissue, Pb tends to settle more in the liver. $(PhSe)_2$ supplementation was effective in prevent the kidney δ-ALA-D inhibition, however, the same was not observed in blood δ-ALA-D activity. Studies considering different experimental models including fish have demonstrated the $(PhSe)_2$ ability to prevent the δ-ALA-D inhibition against the pro-oxidant effects caused by pollutants as metals and pesticides [9-16-28].

Lipid peroxidation caused by mercury exposure can be evaluated by the TBARS formation [29-30]. In our work, a decrease in kidney TBARS levels caused by mercury exposure was observed and this can be attributed to a physiological response due the sub-lethal mercury concentration used in the present study. These results are in agreement with previous studies of our research group, where reduced TBARS levels were observed after mercury and zinc exposure respectively [9, 31]. Pollack et al. (2012) [32] evaluated healthy premenopausal women with low serum levels of cadmium, lead, and mercury and showed that TBARS levels in this population had decreased. Similar results concerning TBARS levels were showed by Zeng et al. 2016 [33] who used a sub-lethal mercury concentration, and observed a decrease in TBARS levels in the liver of large yellow croaker *Pseudosciaena crocea*. The authors attributed this alteration to the significantly increase in the antioxidant enzymes catalase, GR and GST, as an adaptive response. Nevertheless, the exact mechanism that mercury induces oxidative damage is not well understood, but it could be related to alterations in activities of antioxidant enzymes. Furthermore, low levels of mercury can promote a decrease on TBARS levels as showed in literature and in the present study. Thus, the $(PhSe)_2$ preventive effect observed against mercury toxicity may indicate that this

compound acts as an antioxidant. In agreement with this observation, Wilhelm et al. 2009 [34] demonstrated that $(PhSe)_2$ compound has the capacity to reduce ROS generation.

Total thiol and NPSH are the sulfhydryl compounds evaluated in our study. In fact, we observed a decrease in kidney total thiols and NPSH levels in fish exposed to mercury [26]. The ability of mercury to forming complexes with sulfur, thiol, and nitrogen containing ligands frequently is related to the enzyme depletion or inactivation [35]. The (PhSe)2 supplementation not was able to prevent the kidney total thiols depletion. Considering that GSH is the most abundant endogenous non-protein thiol present in all tissues, the non-protein thiol depletion observed in our results can be due to the ability of Hg to bind to GSH. The formation of Hg–GSH complexes is showed in several studies [36-37-38-39].

Ammonia is an important form of nitrogenous waste in *Rhamdia quelen* by renal pathway responsible for 50-84% of the total nitrogen waste excreted [40-41-42]. Mercury exposure did not affect serum ammonia levels, but caused an increase in creatinine levels. The absence of increased ammonia levels could be due to other forms of nitrogen excretion, as through the gills, as described by [43]. The group that received supplemented feeding with (PhSe)2 showed a decrease in creatinine levels indicating better renal condition when compared to the group exposed to mercury chloride and received commercial ration.

In fact, mercury affect renal function, and in the present study, this was not different. We could observe that the kidney had a greater content of Hg compared to the liver. Zn content, also analyzed in this study, has a function in the organism. Animals exposed to Hg presented a decrease of 38.97% in hepatic Zn content and 57.92% in blood Zn content when compared to the control group. Decrease in Zn affects some enzymes activities as δ-ALA-D and could be the reason of inhibition observed. Living organisms have developed homeostatic mechanisms both to ensure the minimum levels required for normal metabolism and to avoid Zn^{2+} intoxication [27-44]. The evident change in Zn homeostasis between the

organs caused by Hg exposure may be responsible for the toxic effects observed, since this balance in the Zn content in the tissues is necessary for the correct functioning of the organism. It is known that Zn^{2+} induces metallothionein synthesis [45-46], and this protein binds to mercury, reducing its availability and toxic effects [47]. The $(PhSe)_2$ supplementation was partially effective to restore Zn homeostasis only in blood tissue. However, this effect was not strong enough to restore the δ-ALA-D enzymatic activity. According to literature, the δ-ALA-D activity depends on the Zn^{2+} presence in its active site [48-49].

In summary, the results demonstrate the ability of Hg to generate nephrotoxic effects in silver catfish, a native species from southern Brazil with a great commercial appeal. The nephrotoxic effects mentioned can be evidenced by the increase in creatinine levels and also by the inhibition of the kidney δ-ALA-D activity. The $(PhSe)_2$ supplementation was able to ameliorate creatinine, which we attributed to the antioxidant effects of this compound. Changes in oxidative parameters, such as total thiols, NPSH and TBARS caused by exposure to Hg were observed in our study. The changes in the oxidative parameters can be the result of the zinc homeostasis alteration between the tissues, since this metal is responsible for the activation of several routes of the antioxidant system in the organisms.

The relation between nephrotoxicity in fish and exposure to Hg remain poorly discussed in the literature. According to the data obtained in this work, damage to renal tissues can occur due to a change in the content of elements such as zinc, which is essential for the body's antioxidant defenses, as well as through the direct interaction of mercury with components of the tissues. The partial but not total prevention obtained by $(PhSe)_2$ supplementation in renal biomarkers can be attributed to the experimental model used which resembles acute mercury intoxication. In fact, 24 hours after the last administration of mercury chloride may be not a sufficient time to occur a greater prevention/recovery of the injured tissues.

Thus, supplementation with $(PhSe)_2$ may be a promising alternative of fish farmers as a preventive agent against the toxic effects of exposure to Hg. However, further investigations are necessary to elucidate the precise mechanism regarding this prevention.

Compliance With Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest

Ethical Approval The approval for the accomplishment of the present study was granted by the committee of ethics in the use of animals of the Federal University of Santa Maria under protocol number 2067170815. The protocol was actualized in July 2018. All the animals (44 male and female of silver catfish) used in the study were treated according to the ethical standards established by the ethics committee in the use of animals and all efforts were made to minimize their suffering.

Acknowledgments:

This study was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Programa PROEX protocol number: 23038.005848/2018-31. Students Tiago da Luz Fiuza and Jossiele Leitemperger received fellowship from CAPES. Vania Lucia Loro received research fellowship from CNPq under process number: 309314/2017-8 .

References

[1] K. Srogi. 2008. Developments in the determination of trace elements by atomic spectroscopic techniques. *Analytical Letters* 41:677 – 724. doi: 10.1080/00032710801934445

[2] Underwood, E.J. 1977. *Trace Elements in Human and Animal Nutrition*. New York, NY: Academic Press.

[3] Pirrone, N., S. Cinnirella, X. Feng, R.B. Finkelman, H.R. Friedli, J. Leaner, R. Mason, A.B. Mukherjee, G.B. Stracher, D.G. Streets, K. Telmer. 2010. Global Mercury emissions to the atmosphere from anthropogenic and natural sources. *Atmos. Chem. Phys.* 10:5951 – 5964. doi: 10.5194/acp-10-5951-2010

[4] WHO. 2003. *Inorganic mercury*. Geneva, Environmental Health Criteria 118: World Health Organization.

[5] Bapu, C., R.C. Purohit, P.P. Sood. 1994. Fluctuation of trace elements during methylmercury toxication and chelation therapy. *Hum. Exp. Toxicol.* 13:815 – 823. doi: 10.1177/096032719401301201

[6] Berlin, M., R.K. Zalups, B.A. Fowler. 2007. *Handbook on the toxicology of metals*. Amsterdam, Boston: Academic Press.

[7] Flohé, L., W.A. Gunzler, H.H. Schock. 1973. Glutathione peroxidase: a selenium enzyme. *FEBS Lett* 32:132 – 134. doi: 10.1016/0014-5793(73)80755-0

[8] Papp, L.V., J. Lu, A. Holmgren, K.K. Khanna. 2007. From Selenium To Selenoproteins: Synthesis, Identity and Their Role In Human Health. *Antioxid. Redox. Signal* 9:775 – 806. doi: 10.1089/ars.2007.1528

[9] Fiuza, T.L., C.S. Oliveira, M. Costa, V.A. Oliveira, G. Zeni, M.E. Pereira. 2015. Effectiveness of $(PhSe)_2$ in protect against the $HgCl_2$ toxicity. *J. Trace Elem. Med. Biol.* 29:255 – 262. doi: 10.1016/j.jtemb.2014.05.008

[10] Mesquita, M., T.F. Pedroso, C.S. Oliveira, V.A. Oliveira, R.F. Santos, C.A. Bizzy, M.E. Pereira. 2016. Effects Of Zinc Against Mercury Toxicity In Female Rats 12 and 48 Hours After $HgCl_2$ Exposure. *EXCLI Journal* 15: 256 – 267. doi: 10.17179/excli2015-709

[11] Barbosa, N.B.V., J.B.T. Rocha, J.C.M. Soares, D.C. Wondracek, J.F. Goncalves, M.R.C. Schetinger, C.W. Nogueira. 2008. Dietary Diphenyl Diselenide Reduces The STZ Induced Toxicity. *Food Chem. Toxicol.* 46:186 – 194. doi: 10.1016/j.fct.2007.07.014

[12] Vargas, N.B.B., C.W. Nogueira, T.N. Guecheva, M.L. Bellinaso, J.B.T. Rocha. 2008. Diphenyl Diselenide Supplementation Delays The Development of N-nitroso-N-methylurea Induced Mammary Tumors. *Arch. Toxicol.* 82:655 – 663. doi: 10.1007/s00204-007-0271-9

[13] Menezes, C.C., J. Leitemperger, A. Santi, T. Lópes, C.A. Vivarberg, S. Peixoto, M.B. Adaime, R. Zanella, N.B.V. Barbosa, V.L. Loro. 2012. The Effect Of Diphenyl Diselenide On Oxidative Stress Biomarkers In *Cyprimus carpio* Exposed To Herbicide Quinclorac (Facet®). *Ecotox. Env. Saf.* 81:91 – 97. doi: 10.1016/j.ecoenv.2012.04.022

[14] Stara, A., J. Kristan, E. Zuskova, J. Velisek. 2013. Effect Of Chronic Exposure To Prometryne On Oxidative Stress And Antioxidant Response In Common Carp (*Cyprinus carpio* L.). *Pestic. Biochem. Physiol.* 10: 518 – 523. doi: 10.1016/j.pestbp.2012.11.002

[15] Santos, C.R., A.L.M. Cavalcante, R.A. Hauser-Davis, R.M. Lopes, R.C.O.C. Mattos. 2016. Effects Of Sub-Lethal And Chronic Lead Concentrations On Blood And Liver ALA-D Activity And Hematological Parameters In Nile Tilapia. *Ecotox. Env. Saf.* 129:250 – 256. doi: 10.1016/j.ecoenv.2016.03.028

[16] Menezes, C., J. Leitemperger, C. Murussi, M.S. Vieira, M.B. Adaime, R. Zanella, V.L. Loro. 2016. Effect Of Diphenyl Diselenide Diet Supplementation On Oxidative Stress Biomarkers In Two Species Of Freshwater Fish Expose To The Insecticide Fipronil. *Fish Physiol. Biochem.* 42:1357 – 1368. doi: 10.1007/s10695-016-0223-5

[17] Baldisserotto, B. 2009. Piscicultura Continental No Rio Grande Sul: Situação Atual, Problemas E Perspectivas Para O Futuro. *Ciência Rural* 39:291 – 299. doi: 10.1590/S0103-84782008005000046

[18] Paulmier, C. 1986. *Selenoorganic Functional Groups.* In: *Paulmier C, editor. Selenium reagents and intermediates in organic synthesis*. Oxford: Pergamon Press.

[19] Sassa, S. 1982. Delta-Aminolevulinic Acid Dehydratase Assay. *Enzyme* 28:133 – 45. doi: 10.1159/000459097

[20] Thomas, L. 1998. *Alanine Aminotransferase (ALT), Aspartate Aminotranferase (AST).* In: *L. Thomas (Ed.), Clinical Laboratory Diagnostics, 1st ed*. Frankfurt: Verlangsgesellschaft. TH-Books.

[21] Ohkawa, H., N. Ohishi, K. Yagi. 1979. Assay For Lipid Peroxides In Animal-Tissues By Thiobarbituric Acid Reaction. *Anal. Biochem.* 95:351 – 358. doi: 10.1016/0003-2697(79)90738-3

[22] Ellman, G.L. 1959. Tissue Sulfhydryl Groups. *Arch. Biochem. Biophys.* 82:70 – 77. doi: 10.1016/0003-9861(59)90090-6

[23] Ineu, R.P., C.S. Oliveira, V.A. Oliveira, L. Moraes-Silva, S.C.A. Luz, M.E. Pereira. 2013. Antioxidant Effect Of Zinc Chloride Against Ethanol-Induced Gastrointestinal Lesions In Rats. *Food Chem. Toxicol.* 58:522 – 529. doi: 10.1016/j.fct.2013.05.022

[24] Bradford, M.M. 1976. A Rapid And Sensitive Method For The Quantitation Of Microgram Quantities Of Protein Utilizing The Principle Of Protein-Dye Binding. *Anal. Biochem.* 72:248 – 254. doi: 10.1016/0003-2697(76)90527-3

[25] Farina, M., R. Brandão, F.S. Lara, L.B. Pagliosa, F.A. Soares, D.O. Souza, J.B.T Rocha. 2003. Profile Of Nonprotein Thiols, Lipid Peroxidation And Delta-Aminolevulinate Dehydratase Activity In Mouse Kidney And Liver In Response To Acute Exposure To Mercuric Chloride And Sodium Selenite. *Toxicology* 184:179 – 187. doi: 10.1016/S0300-483X(02)00576-0

[26] Ynalvez, R., J. Gutierrez, H. Gonzalez-Cantu. 2016. Mini-review: Toxicity Of Mercury As A Consequence Of Enzyme Alteration. *Biometals* 29:781 – 788. doi: 10.1007/s10534-016-9967-8

[27] Bernard, A., R. Lauwerys. 1987. Metal-Induced Alterations of Aminolevulinic Acid Dehydratase. *Ann. NY. Acad. SCI.* 514: 41 – 47. doi: 10.1111/j.1749-6632.1987.tb48759.x

[28] Luchese, C., R. Brandão, R. Oliveira, C.W. Nogueira, F.W. Santos. 2007. Efficacy Of Diphenyl Diselenide Against Cerebral And Pulmonary Damage Induced By Cadmium In Mice. *Toxicol. Lett*. 173:181 – 190. doi: 10.1016/j.toxlet.2007.07.011

[29] Fukino, H., M. Hirai, Y.M. Hsueh, Y. Yamane. 1984. Effect of zinc pretreatment on mercuric chloride- induced lipid peroxidation in the rat kidney. *Toxicol. Appl. Pharmacol*. 73:395 – 401. doi: 10.1016/0041-008X(84)90091-7

[30] Hussain, S., A. Atkinson, S.J. Thompson, A.T. Khan. 1999. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver and kidneys of mice. *J. Environ. Sci. Health* 34:645 – 60.doi: 10.1080/03601239909373219

[31] Leitemperger, J., C. Menezes, A. Santi, C. Murussi, T. Lopes, M. Costa, L.S. Nogueira, V.L. Loro. 2016. Early Biochemical Biomarkers For Zinc In Silver Catfish (*Rhamdia quelen*) After Acute Exposure. *Fish Physiol. Biochem*. 42:1005 – 1014. doi: 10.1007/s10695-015-0192-0

[32] Pollack, A.Z., E.F. Schisterman, L.R. Goldman, S.L. Mumford, N.J. Perkins, M.S. Bloom, C.B. Rudra, R.W. Browne, J. Wactawski-Wende. 2011. Relation of blood cadmium, lead, and mercury levels to biomarkers of lipid peroxidation in premenopausal women. *Am. J. Epidemiol.* 175(7):645 – 652. doi: 10.1093/aje/kwr375

[33] Zeng, L., J.L. Zheng, Y.H. Wang, M.Y. Xu, A.Y. Zhu, C.W. Wu. 2016. The Role of Nrf2/Keap 1 Signaling In Inorganic Mercury Induced Oxidative Stress In The Liver Of Large Yellow Croaker *Pseudosciaena crocea*. *Ecotox. Environ. Saf.* 132:345 – 352. doi: 10.1016/j.ecoenv.2016.05.002

[34] Wilhelm, E.A., C.R. Jesse, M.R. Leite, C.W. Nogueira. 2009. Studies On Preventive Effects Of Diphenyl Diselenide On Acetaminophen-Induced Hepatotoxicity In Rats. *Pathophysiology* 16:31 – 37. doi: 10.1016/j.pathophys.2008.12.002

[35] Tinkov, A.A., O.P. Ajsuuvakova, M.G. Skalnaya, E.V. Popva, A.I. Sintiskii, O.N. Nemershina, E.R. Gatiatulina, A.A. Nikonorov, A.V. Skalny. 2015. Mercury And Metabolic Syndrome: A Review Of Experimental And Clinical Observations. *Biometals* 28:231 – 254. doi: 10.1007/s10534-015-9823-2

[36] Rooney, J.P. 2007. The Role Of Thiols, Dithiols, Nutritional Factors And Interacting Ligands In The Toxicology Of Mercury. *Toxicology* 234:145 – 156. doi: 10.1016/j.tox.2007.02.016

[37] Lu, S.C. 2013. Glutathione Synthesis. *Biochem. Biophys. Acta.* 1830:3143 – 3153. doi: 10.1016/j.bbagen.2012.09.008

[38] Elia, A.C., R. Galarini, M.I. Taticchi, A.J.M. Dörr, L. Mantilacci. 2003. Antioxidant Responses And Bioaccumulation In *Ictalurus melas* Under Mercury Exposure. *Ecotoxicol. Environ. Saf.* 55:162 – 167. doi: 10.1016/S0147-6513(02)00123-9

[39] Brandão, F., T. Cappello, J. Raimundo, M.A. Santos, M. Maisano, A. Mauceri, M. Pacheco, P. Pereira. 2015. Unravelling The Mechanisms Of Mercury Hepatotoxicity In Wild Fish (*Liza aurata*) Through A Triad Approach: Bioaccumulation, Metabolomic Profiles And Oxidative Stress. *Metallomics* 7:1352 – 1363. doi: 10.1039/c5mt00090d

[40] Altinok, I., J.M. Grizzle. 2004. Excretion Of Ammonia and Urea By Phylogenetically Diverse Fish Species in Low Salinities. *Aquaculture* 238:499 – 507. doi: 10.1016/j.aquaculture.2004.06.020

[41] Kajimura, M., S.J. Croke, C.N. Glover, C.M. Wood. 2004. Dogmas and Controversies In The Handling of Nitrogenous Wastes: The Effect of Feeding and Fasting on The Excretion of Ammonia, Urea and Other Nitrogenous Waste Products In Rainbow Trout. *J. Exp. Biol.* 207:1993 – 2002. doi: 10.1242/jeb.00901

[42] Lam, S.S., A. Jusoh, A.T. Law. 2008. Waste Excretion Of Marble Goby (*Oxyeleotris marmorata* Bleeker) Fed With Different Diets. *Aquaculture* 274:49 – 56. doi: 10.1016/j.aquaculture.2007.11.023

[43] Wright, P.A., C.M. Wood, J. Hiroi, J.M. Wilson. 2016. (Uncommon) Mechanisms Of Branchial Ammonia Excretion In Common Carp (*Cyprinus carpio*) in Response To Environmentally Induced Metabolic Acidosis. *Physiological and Biochemical Zoology* 89:26 – 40. doi: 10.1086/683990

[44] Pagani, A., L. Villarreal, M. Capdevila, S. Atrian. 2007. The *Saccharomyces cerevisiae* Crs5 Metallothionein Metal-Binding Abilities and its Role in The Response To Zinc Overload. *Mol. Microbiol.* 63: 256 – 269. doi: 10.1111/j.1365-2958.2006.05510.x

[45] Peixoto, N.C., T. Roza, E.M. Flores, M.E. Pereira. 2003. Effects of Zinc and Cadmium on $HgCl_2$-Delta-ALA-D Inhibition And Hg Levels in Tissues Of Suckling Rats. *Toxicol. Lett.* 46:17 - 25. doi: 10.1016/j.toxlet.2003.08.006

[46] Peixoto, N.C., M.A. Serafim, E.M.M. Flores, M.J. Bebianno, M.E. Pereira. 2007. Metallothionein, Zinc, and Mercury Levels in Tissues Of Young Rats Exposed To Zinc and Subsequently To Mercury. *Life Sci.* 81:1264 - 1271. doi: 10.1016/j.lfs.2007.08.038

[47] Dabrio, M., A.R. Rodriguez, G. Bordin, M.J. Bebianno, M.D. Ley, I. Sestakova. 2002. Recent Developments in Quantification Methods For Metallothionein. *J. Inorg. Biochem.* 88:123 – 34. doi: 10.1016/S0162-0134(01)00374-9

[48] Godwin, H.A. 2001. The Biological Chemistry Of Lead. *Curr. Opin. Chem. Biol.* 5:223 – 227. doi: 10.1016/S1367-5931(00)00194-0

[49] Jaffe, E.K. 1995. Porphobilinogen Synthase, The First Source of Heme's Asymmetry. *J. Bioen. Biomem.* 27:169 – 179. doi: PMID7592564

Subtitles

Fig. 1. Liver (A), kidney (B) and blood (C) δ-ALA-D activity. Female and male silver catfish were treated with $(PhSe)_2$ supplemented feed (3.0 mg/kg/day) or commercial feed during thirty days, and exposed to $HgCl_2$ (1.7 mg/kg/day, i.p.) or saline in the last five days. The results are expressed as mean ± S.E.M. Duncan's multiple range test: groups statistically equals are represented by the same letters and groups statistically different are represented by different letters ($p \leq 0.05$; n = 6–8).

Fig. 2. Liver (A) and kidney (B) TBARS levels from silver catfish treated as described in Fig. 1. The results are expressed as mean ± S.E.M. Duncan's multiple range test: groups statistically equals are represented by the same letters, and groups statistically different are represented by different letters ($p \leq 0.05$; n = 6–8).

Fig. 3. Liver (A) and kidney (B) total thiols levels from silver catfish treated as described in Fig. 1. The results are expressed as mean ± S.E.M. Duncan's multiple range test: groups statistically equals are represented by the same letters, and groups statistically different are represented by different letters ($p \leq 0.05$; n = 6–8).

Fig. 4. Liver (A) and kidney (B) NPSH levels from silver catfish treated as described in Fig. 1. The results are expressed as mean ± S.E.M. Duncan's multiple range test: groups statistically equals are represented by the same letters, and groups statistically different are represented by different letters ($p \leq 0.05$; n = 6–8).

Figure 1

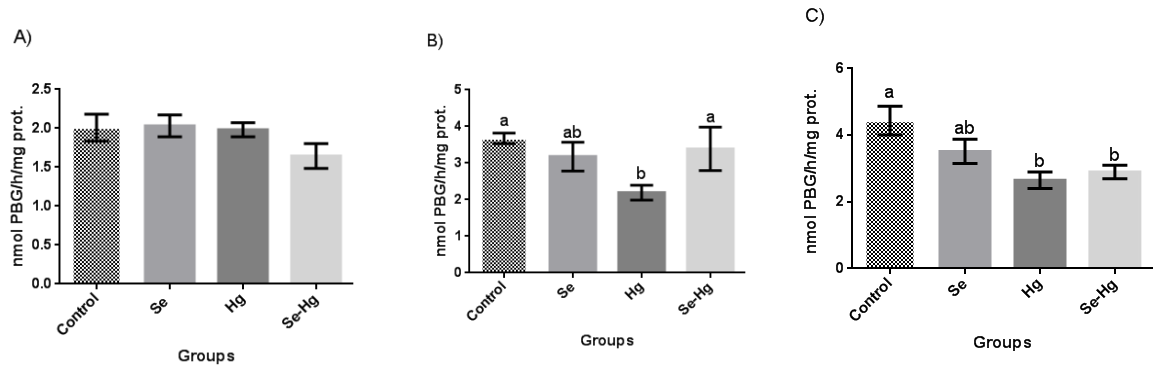


Figure 2

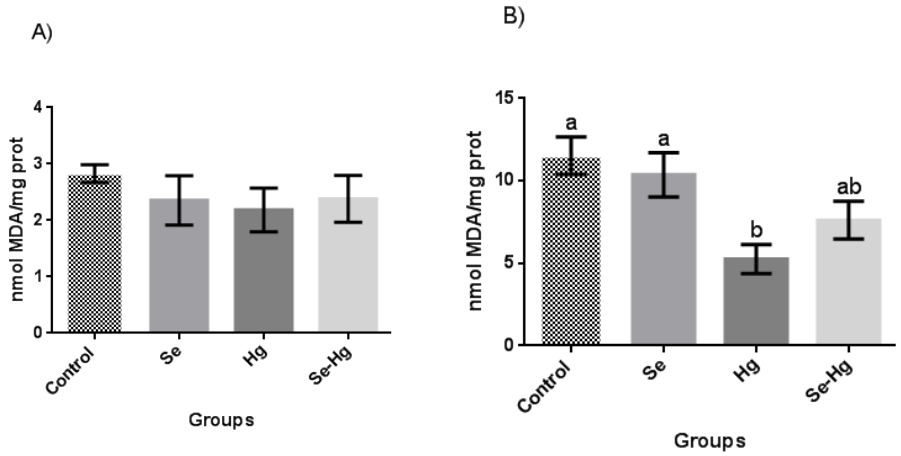


Figure 3

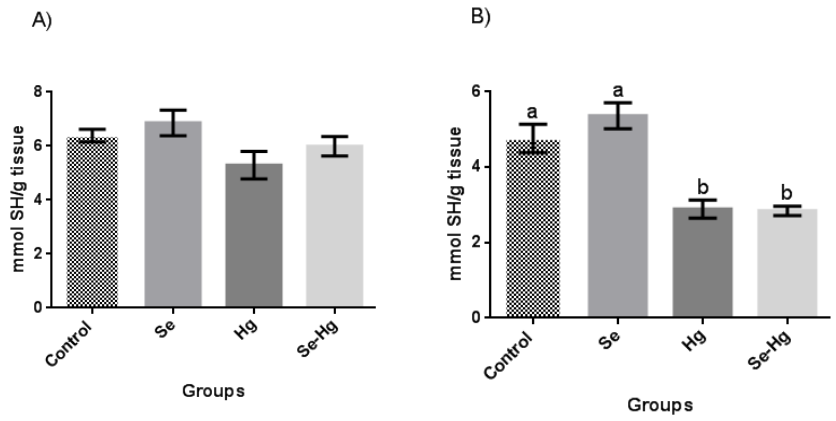


Figure 4

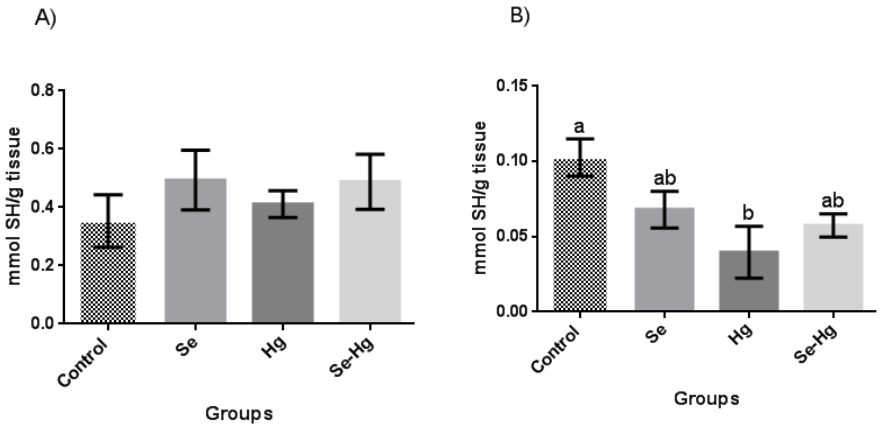


Table 1

Plasmatic hepatic (AST and ALT) and renal (Ammonia and Creatinine) toxicity parameters from silver catfish treated during thirty days with $(PhSe)_2$ supplemented feed (3.0 mg/kg) or control feed and exposed (i.p) to $HgCl_2$ (1.7 mg/kg) or Saline (0.9%) for 5 days.

Group	**ALT (U/L)**	**AST (U/L)**	**Ammonia (mg/dL)**	**Creatinine (mg/dL)**
Control	70.57±14.29	31.90±6.22	15.19±1.27	$0.083±0.007^{a}$
Se	80.18±12.87	36.49±8.47	18.15±1.72	$0.077±0.025^{a}$
Hg	65.50±13.72	30.98±6.43	17.95±1.41	$0.16±0.016^{b}$
Se-Hg	74.48±12.87	38.05±6.55	16.29±1.53	$0.11±0.026^{ab}$

Data are expressed as mean ± S.E.M. (n = 6 - 8) and the values accompanied by different letters in the same column are statistically different ($p \leq 0.05$).

Table 2

Hg and Zn levels in liver, kidney and blood from silver catfish treated during thirty days with $(PhSe)_2$ supplemented feed (3.0 mg/kg) or control feed and exposed (i.p) to $HgCl_2$ (1.7 mg/kg) or Saline (0.9%) for 5 days.

Group	Liver	Kidney	Blood
Hg levels (µg Hg/g tissue)			
Control	0.42±0.04[a]	0.19±0.07[a]	nd
Se	0.08±0.02[a]	0.33±0.08[a]	nd
Hg	7.49±1.61[b]	10.69±1.13[b]	nd
Se-Hg	5.51±2.18[b]	10.60±1.63[b]	nd
Zn levels (µg Zn/g tissue)			
Control	17.96±0.84[a]	20.15±0.56	3.85±0.72[a]
Se	20.18±1.69[a]	24.18±3.50	3.17±0.33[ab]
Hg	10.96±0.87[b]	17.95±4.95	1.62±0.18[b]
Se-Hg	13.41±0.76[b]	19.65±1.99	2.62±0.53[ab]

Data are expressed as mean ± S.E.M. (n = 4) and the values accompanied by different letters in the same column are statistically different ($p \leq 0.05$). The sample whose mercury concentration was below the detectable limit of the technique are represented by nd (non-detected).

5. MANUSCRITO

Protective effect of diphenyl diselenide $(PhSe)_2$ supplementation on $HgCl_2$ toxicity in mice

Tiago da Luz Fiuza[1,2]; Luciana Joner Guerra[1,2]; Jossiele Weiss Leitemperger[12]; Jamile da Silva Bernardi[2]; Aline Teixeira Marins[13]; Eduardo Stringini Severo[13]; Maria Ester Pereira[2]; Vania Lucia Loro[123]

[1]Laboratory of Aquatic Toxicology, 2Graduate Program in Biological Sciences: Toxicological Biochemistry, 3Animal Biodiversity, Natural and Exact Sciences Center, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil.

* Correspondence to:
Vania Lucia Loro
Department of Biochemistry and Molecular Biology, Natural and Exact Sciences Center, Federal University of Santa Maria. 1000 Roraima Avenue, Santa Maria, RS, 97105-900, Brazil. E-mail: vania.loro@gmail.com

Abstract

This work investigated the effects of diet containing diphenyl diselenide [$(PhSe)_2$] against the toxic effects of mercury in mice (Swiss Albino). The animals were treated during 30 consecutive days with a $(PhSe)_2$ supplemented diet (3.0 mg kg^{-1}) or commercial diet. During the last 5 days the animals received a daily subcutaneous injection of $HgCl_2$ (5.0 mg kg^{-1}) or Saline (0.9%). Twenty-four hours after the last $HgCl_2$ injection, the animals were euthanized by cardiac puncture and tissues collected. Hepatic (AST and ALT) and renal (urea and creatinine) markers, δ-ALA-D activity, TBARS, total and non-protein thiols levels as well as hepatic, renal and blood mercury (Hg) and zinc (Zn) content were evaluated. Considering renal parameters, $HgCl_2$ exposure increased serum urea and creatinine levels and non-protein thiols levels. There was a TBARS and total thiols levels decrease. $HgCl_2$ exposure also decreased blood δ-ALA-D activity. The $(PhSe)_2$ supplementation totally prevented mercury induced alterations in blood δ-ALA-D activity, urea and creatinine levels and total thiols levels. Animals exposed to $HgCl_2$ presented an increase in liver and kidney Hg content and an increase in kidney Zn content. The kidney accumulated more compared with liver. The $(PhSe)_2$ supplementation did not statistically alter Hg tissue distribution. No effects of $HgCl_2$ exposure on hepatic tissue were observed. These results show that the nephrotoxic effects presented by Hg exposure can be prevented by $(PhSe)_2$ diet supplementation in mice.

Keywords: Nephrotoxicity; Inorganic mercury; Selenium;

Introduction

The toxicity of mercury (Hg) and toxicological risks have been extensively investigated and debated. Coming from natural sources and/or anthropogenic this metal can be found in terrestrial, aquatic and atmospheric ecosystems (Streets et al., 2011; (Carocci et al., 2014; Sheehan et al., 2014). Hg can be found in elemental, organic and inorganic forms and is a non essential element for living organisms (Li et al., 2009; WHO, 2003). The mercury toxicity is closely linked to the form of this metal. Nephrotoxicity being related to the inorganic form of this metal while damage to the central nervous system (CNS) and hepatic tissue related to the organic form (Counter and Buchanan, 2004; Graeme and Pollack, 1998). In addition, studies have shown that rats exposed to inorganic mercury (mercury chloride - $HgCl_2$) had renal dysfunction evidenced by elevated levels of urea and creatinine, once, this metal has high affinity for -SH groups of endogenous biomolecules (Clarkson, 1997; Peixoto and Pereira, 2007). Therefore, essential enzyme systems, for example the sulfhydryl δ-ALA-D enzyme may have their activities inhibited by the action of Hg (Emanuelli et al., 1996; Rocha et al., 1995). Several studies have been carried out looking for alternatives to reducing Hg damage (Agarwal et al., 2010; Rooney, 2007). In this way, organic elements as selenium could be a good alternative. Fish poisoning with mercury chloride and feeding before with diet containing diphenyl diselenide show a better.

Selenium (Se) is a chemical element belonging to group 16 of the periodic table and also recognized as an essential micronutrient for the human body. Considering the beneficial effects previously described about the Se atom, researchers turned their attention to organoselenium compounds synthesis looking for an innovative proposals focusing on their therapeutic use (Ferreira et al., 2018). The most recognized roles of selenium in metabolism is your presence in the active site of the enzyme glutathione peroxidase (GPx) thus making it an important element for the antioxidant system. Selenium also display a protective properties against Hg toxicity (Barbosa et al., 2017; Barceloux, 1999; Kuraś et al., 2018). Diphenyl diselenide $(PhSe)_2$ is an organic Se compound with antioxidant properties, probably due to ability to mimetizing the GPx enzyme (Meotti et al., 2004; Silvestre et al., 2014). In addition, $(PhSe)_2$ protects against hematological changes and renal damage caused by Hg exposure in mice, as well as alters the distribution of this metal in tissues of rodents (Brandão et al., 2008; Fiuza et al., 2015). The antioxidant activity of $(PhSe)_2$ in the body can be attributed to its

ability to induce the synthesis of endogenous antioxidants such as metallothioneins and glutathione (GSH) (de Bem et al., 2013; Brandão et al., 2006).

Therefore, considering the Hg risks for living organisms and the mechanisms involved to its toxicity, this work aimed to evaluate the preventive effects of a $(PhSe)_2$ supplemented diet against the damage caused by mercury chloride exposure. Hepatic, renal and blood toxicity markers were evaluated as well as mercury and zinc tissue distribution using rodents as experimental model.

Materials and Methods

Chemicals

The $(PhSe)_2$ used in present study was obtained by synthesis in accordance with Paulmier (1986). $(PhSe)_2$ chemical purity (99.9%) was determined by GC/HPLC. Mercury chloride (99.5% chemical purity) was purchased from Merck (Darmstadt, Germany). The commercial kits used to determine the AST, ALT, Urea and creatinine levels were obtained from Labtest Diagnóstica S.A (Lagoa Santa, MG Brazil).

Animals

Animals used in our experiment were obtained from the breeder at the Federal University of Santa Maria. Adult male Swiss mice weighing 20 – 25g were transferred to our laboratory and kept for one week to acclimatization. During the experimental period the animals were maintained on 12 hours of light/dark cycle, 22°±2°C temperature and free access to water and control or supplemented feed depending on the experimental group. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil (Process number: 2067170815). All efforts were made to minimize the number of animals used and their suffering.

Experimental protocol

Animals were treated during 30 consecutive dais with control or $(PhSe)_2$ (3.0mg/kg) supplemented ration. After 25 days of treatment the animals received one dose daily of $HgCl_2$ (5.0 mg/Kg) or saline (0.9% NaCl) by subcutaneous injection during the last five days of treatment. Animals were distributed in four experimental groups: Control (control feed +

saline); Selenium ($(PhSe)_2$ supplemented feed + saline); Mercury (Control feed + $HgCl_2$); Selenium-Mercury ($(PhSe)_2$ supplemented feed + $HgCl_2$). Twenty four hours after the last administration of $HgCl_2$ or saline injection, animals were euthanized by cardiac puncture and blood, renal and hepatic tissues samples were collected for subsequent biochemical analysis.

Tissues preparation

Blood samples without anticoagulant were collected and centrifuged at 3000 rpm to obtain the serum for alanine aminotrasferase (ALT), aspartate aminotrasferase, urea and creatinine determination. Another part of blood samples containing anticoagulant was diluted in distilled water in 1:5 proportions under constant agitation in ice bath during 10 min to obtain the samples for blood δ-ALA-D determination. Kidney and liver samples were quickly removed and placed in ice to subsequent homogenization in 5 and 7 volumes respectively of Tris-HCl buffer (10mM, pH 7.4) with 10 up-and-down strokes at 1200 rpm in a Teflon-glass homogenizer. The homogenate obtained was centrifuged at 3000 × g at 4 ◦C for 20 min and the supernatant fraction (S1) was used in the kidney and liver δ-ALA-D and oxidative parameter determination. Furthermore, approximately 500 mg of kidney and liver, 500µL of blood were kept at -20° until the Hg and Zn determination.

δ-ALA-D activity determination

The determination of δ-ALA-D was performed as described by Peixoto et al., (2003) and Sassa, (1982). The incubation at 39°C was performed during 30, 60 and 90 min for liver, blood and kidney respectively using 100 µl of S1 for liver and 200 µl for blood or kidney. Trichloroacetic acid 10% (TCA) containing $HgCl_2$ 0.05M was used to stop the enzymatic reaction and the final product of the reaction porphobilinogen (PBG) was determined spectrophotometrically at 555nm through the addiction of Ehrlich's reagent using the molar absorption coefficient of the Ehrlich-PBG salt (6.1X104). The specific enzymatic activity was expressed as nmol PBG/h/mg protein.

ALT and AST activity determination

The determination of ALT and AST activity was carried out similarly with only difference in the substrate used, 83.3 mM L-alanine acid for ALT and 83.3 mM L-aspartic acid for AST such as previously described in Thomas (1998). The incubation medium containing Tris-HCl buffer 55.8 mM pH 7.15, -ketoglutaric acid 1.67 mM, sodium azide 12.8

mM was obtained from Kovalent commercial kit and used to determine both enzyme activities and the specific enzymatic activity was expressed in U/L.

Urea Levels

To urea levels determination. 10 µL of serum sample was added to a medium containing phosphate buffer (19.34 mM pH 6.9), sodium salicylate (58.84 mM), sodium nitroprusside (3.17 mM), and urease (≥12.63 UK/L) for 5 min at 39 ◦C. The reaction was stopped by adding oxidant solution (final concentrations: NaOH 0.07 M and sodium hypochlorite 3.01 mM) and incubated for another 5 min. The urea hydrolyzed by the urease enzyme produces ammonium ions and CO_2. The ammonium ions react in alkaline medium with salicylate and hypochlorite under the catalytic action of sodium nitroprusside to form the indophenol blue (a colored product). The color intensity determined spectrophotometrically at 600 nm is proportional to urea levels (mg/dL) in the sample.

Creatinine Levels

Serum sample was added to an incubation medium containing picric acid (20.2 mM) and NaOH (145.4 mM) and incubated at 37°C. Creatinine picrate is formed when creatinine react with picric acid and spectrophotometrically determined at 510 nm. The results were expressed in mg of creatinine per dL of serum.

Lipid peroxidation measurement

Lipid peroxidation was determined through thiobarbituric acid-reactive species (TBARS) technique described by Ohkawa et al. (1979). Hepatic and renal S1 were incubated at 95°C in an incubation medium containing thiobarbituric acid (TBA) (0.8%), sodium dodecyl sulfate SDS (8.1%) and acetic acid buffer (2.5 M, pH 3.4) during 2 hours. A curve using malondialdehyde (MDA) (0, 0.15, 0.3, 0.6, 1.2 and 1.5 nmol MDA) as standard was constructed in order to express the results in nmol MDA/mg protein and the absorbance was measured spectrophotometrically at 532 nm.

Total thiols and non-protein thiols levels determination

Kidney and liver S1 were used for total thiols levels determination using the DTNB 10mM as a chromogen (Ellman, 1959). Liver and kidney S1 were added to TCA 4% in a 1:1 proportion and centrifuged at 2000 x g for 10 min to obtain the S2 precipitate which was used

to determinate the non-protein thiols levels. A standard curve using glutathione was constructed in order to calculate the SH in the tissue samples. The thiol levels were expressed as nmol SH/mg protein.

Metal levels determination

Coupled plasma atomic emission (ICPE-9000 Shimadzu Scientific Instruments) was used for metal levels determination. Liver, kidney and blood samples were collected and transferred to individual vials and kept at -20°C for subsequent digestion. The digestion was performed using distilled HNO^3 (12.5 ml/1 g of tissue) in boiling water bath (100°C) until no solid particles of tissue were observed (8 hours approximately). The analytical Hg and Zn standard (Merck®) was used to construct the standard curve (0, 50, 150, 300, 600, 1200 ppb) (r = 0.99334) and (0, 10, 30, 60, 120, 240 ppb) (r = 0.99614), respectively.

Protein content determination

The protein content was determined using Coomassie blue according to Bradford (1976) using bovine serum albumin as a standard.

Statistical analysis

The data obtained were analyzed by one-way ANOVA followed by Duncan´s multiple range test to obtain the differences among groups. The significant difference among groups was established when $p \leq 0.05$ and are represented in the graphics by different letters.

Results

δ-ALA-D Activity

Liver, kidney and blood are shown in figure 1. δ-ALA-D activity from renal and hepatic tissues was not affected by $(PhSe)_2$ or mercury treatment. A decrease in blood ALA-D activity was observed. Animals exposed to mercury presented a significant blood ALA-D activity inhibition and this alteration in blood ALA-D activity was totally prevented by $(PhSe)_2$ feed supplementation (Duncan $p \leq 0.05$).

Serum urea and creatinine levels

Urea and Creatinine levels are shown in figure 2. Mercury exposures increase significantly serum urea and creatinine levels. $(PhSe)_2$ feed supplementation was effective to protect against this increase (Duncan $p \leq 0.05$).

AST and ALT activity

AST and ALT activity are shown in figure 3. AST activity was not affected by $(PhSe)_2$ supplementation and mercury exposure. One way ANOVA revealed a significant alteration in ALT activity caused by mercury exposure. A significant decrease in ALT activity was observed in the mercury group compared to control group (Duncan $p \leq 0.05$). The $(PhSe)_2$ supplementation partially prevented the observed ALT activity alteration.

Total thiols and non-protein thiols levels

Liver and kidney total and non-protein thiols levels are shown in figure 4. Animal that received mercury treatment presented a significant decrease in kidney total thiols levels but the same not was observed on the total thiols from liver. Animals that received the combination of $(PhSe)_2$ supplementation and mercury presented a significant increase in liver total thiols levels and a protection against the mercury effects in kidney. One way ANOVA reveal an alteration in kidney non-protein thiols levels caused by mercury exposure, however liver non-protein thiols levels were not affected by mercury exposure. A significant increase in kidney non-protein thiols levels was observed in animals exposed to mercury. $(PhSe)_2$ supplementation was not effective to protect against the increase in kidney non-protein thiols levels (Duncan $p \leq 0.05$).

TBARS levels

Liver (A), kidney (B) TBARS levels are shown in figure 5. One way revealed a significant decrease in kidney TBARS levels in animals exposed to mercury, and $(PhSe)_2$ supplemented feed was not effective in protecting against this decrease. Liver TBARS levels not were affected by any treatment realized. $(PhSe)_2$ supplemented feed alone did not cause any change in kidney and liver TBARS levels.

Hg and Zn levels

The groups exposed to Hg presented an increase in Hg levels in kidney and liver (Duncan $p \leq 0.05$). The kidneys from animals of both groups exposed to Hg presented a higher Hg concentration when compared with the liver of the same animals. Animals than received $(PhSe)_2$ supplemented feed presented a tendency, but not statistically significant, to reduce the kidney and liver Hg levels. The both groups exposed to Hg did not alter in blood and liver Zn content. A statistically significant increase in kidney Zn content was observed in both groups exposed to Hg and the $(PhSe)_2$ supplementation not was effective to protect against this alteration. $(PhSe)_2$ supplementation per se did not cause any alteration in Zn content of all tissues evaluated.

Discussion

This study investigate the feed $(PhSe)_2$ supplementation effects against Hg exposure evaluating toxicity markers in different tissues (δ-ALA-D), hepatic and renal damage markers, oxidative parameters besides Hg and Zn levels to evaluate the metal homeostasis through the organs. The Hg toxicity is related with its –SH radical affinity, thereby an important finding of in our study is the blood ALA-D activity inhibition because this enzyme is dependent of the -SH stabilization in your active site. Besides not significant, a reduction in kidney ALA-D activity was observed in our study which can evidenced the Hg presence in this organ. Previous studies conducted by our research group demonstrated Hg ability to inhibit blood and kidney ALA-D activity in different animal models as mammals and fish (Fiuza et al., 2015; Oliveira et al., 2014; Oliveira et al., 2015; Mesquista et al., 2016; da Luz Fiuza et al., 2018). Diphenyl diselenide in the present study could be a potential antioxidant. In fact we observed an improvement in blood ALA-D activity in the mice that received the $(PhSe)_2$ supplementation and were exposed to Hg. These observed results are in agreement with other studies using $(PhSe)_2$ as a protector agent against the toxic effects of different inhibitors of the ALA-D activity, including Hg (Menezes et al., 2012; Fiuza et al., 2015; da Luz Fiuza et al., 2018). We attributed the protective effects to $(PhSe)_2$ antioxidant effects previously mentioned.

TBARS is a general marker of tissue injury. The decrease in kidney TBARS levels observed in our study does not represent a tissue lesion but a possible adaptive response caused by a change in the antioxidant system which is responsible for the xenobiotics

elimination. Administration of 1.0 mg / kg of mercury chloride in mice for two weeks, unlike our study, the authors observed an increase in TBARS levels (Brandão et al., 2009). However, the results found in the present study are in agreement with other results found by our study group (Fiuza et al., 2015; Leitemperger et al., 2016; da Luz Fiuza et al., 2018). This difference between results demonstrates that the ability of mercury chloride to induce tissue damage depends not only the presence of this metal in the body but also depends on the dose administered and time of exposure. As mentioned before Hg have a great sulphydryl affinity. Low-molecular-weight thiols such as cysteine, homocysteine (Hcy), and glutathione (GSH) are metabolites of the sulfur cycle in biological systems (Meister, 1988). NPSH in the body (95%) also represents an important pathway in the cellular defense system due to its reducing/antioxidant properties and toxic compound inactivation (Lu, 2013; Meister, 1988; Rooney, 2007). The increase in NPSH levels observed in present work could be related with the ability of low or non-toxic inorganic Hg dose in stimulate increase GSH synthesis as showed by Zalups, 2000. The treatment with $(PhSe)_2$ supplementation was effective to increase NPSH levels that represents a good alternative against mercury toxicity. The decrease in kidney total thiols levels observed in the present study are in accordance with results already described (Oliveira et al., 2015) and can be explained due to the fact that Hg cause renal injury, such as cell detachment, tubular necrosis and cell vacuolation and these damages in the renal cells lead to intense proteinuria (Hazelhoff et al., 2018). However the group that received $(PhSe)_2$ supplementation presented an total protection against the decrease in kidney total thiols levels.

It is know that the inorganic mercury cause renal damage which can lead to renal dysfunction (Brandão et al., 2010; Moraes-Silva et al., 2012a; Peixoto et al., 2007). Several studies conducted by our research group demonstrated Hg nephrotoxicity in different animal species, poisoning model, administration route, different doses and exposure time (Fiuza et al., 2015; da Luz Fiuza et al., 2018; Mesquita et al., 2016; Moraes-Silva et al., 2012a; Oliveira et al., 2014, 2015; Peixoto et al., 2007). The increase in the markers creatinine and urea levels observed in present study indicates renal impairment, which was totally prevented by $(PhSe)_2$ supplementation. The $(PhSe)_2$ protection against the toxic effects of Hg in renal and other tissue are in accordance with literature (Brandão et al., 2008; Fiuza et al., 2015; da Luz Fiuza et al., 2018). Another toxic-markers evaluated in our study were AST and ALT activities. $(PhSe)_2$ supplementation was effective to protect against ALT activity inhibition observed in the present study. The observed inhibition is related to the direct interaction between Hg and

sulfhydryl groups present in the ALT structure. Our research group reported in other studies the inhibition of ALT activity caused by exposure to Hg (Fiuza et al., 2015; Franciscato et al., 2011; Peixoto and Pereira, 2007).

Concerning organs that accumulated Hg the kidney was the organ that presented the highest levels of this metal (4.8 folds more than liver). Kidney classically is the organ that accumulates more inorganic Hg. These results are in agreement with previous works from our research group (Favero et al., 2014; Fiuza et al., 2015; Franciscato et al., 2011; da Luz Fiuza et al., 2018; Mesquita et al., 2016; Oliveira et al., 2014; Peixoto et al., 2003). However, in the present study Hg accumulation in kidneys was not prevented by $(PhSe)_2$ supplementation. Different of mercury, zinc had several functions in the body, being one of these functions its ability to induce metallothionein synthesis. Both groups exposed to Hg presented an increase in kidney Zn content, suggesting a possible try to improve the metallothionein syntheses in kidney to eliminate Hg or make it inactive.

In this research, we demonstrated the ability of a supplementation with $(PhSe)_2$ in protecting against inhibition in blood δ-ALA-D and ALT activities. The mechanism involved in δ-ALA-D and ALT activities improvement can be attributed to the ability of (PhSe)2 to induce the kidney metalothionein synthesis as reported previously by Brandão et al. (2006). Thus, the metalothionein synthesized can react with circulating Hg in the bloodstream and conduce Hg to renal elimination. Another important point observed in our study was the improvement in renal status of the animals that received $(PhSe)_2$ in the diet and were exposed to Hg. This improvement in renal function may be noted by the return of the urea and creatinine to control levels and also by the increase of total thiols levels of the kidneys, which indicates lower loss of protein content through the kidneys. The results obtained show that dietary supplementation with $(PhSe)_2$ could be a promising alternative to protect against kidney damage caused by mercury exposure due to ability of this selenium compound to enhance antioxidant defenses. In addition, other studies considering other toxic agents with mercury-like toxicity can be carried out further exploring the possible protective effect of $(PhSe)_2$ against the toxicity of environmental pollutants.

Competing interests

The authors declare that no competing interests exist.

References

Agarwal, R., Goel, S.K., Chandra, R., and Behari, J.R. (2010). Role of vitamin E in preventing acute mercury toxicity in rat. Environ. Toxicol. Pharmacol. 29, 70–78.

Barbosa, N.V., Nogueira, C.W., Nogara, P.A., de Bem, A.F., Aschner, M., and Rocha, J.B.T. (2017). Organoselenium compounds as mimics of selenoproteins and thiol modifier agents. Met. Integr. Biometal Sci. 9, 1703–1734.

Barceloux, D.G. (1999). Selenium. J. Toxicol. Clin. Toxicol. 37, 145–172.

Bechara, E.J. (1996). Oxidative stress in acute intermittent porphyria and lead poisoning may be triggered by 5-aminolevulinic acid. Braz. J. Med. Biol. Res. Rev. Bras. Pesqui. Medicas E Biol. 29, 841–851.

Bechara, E.J.H., Medeiros, M.H.G., Monteiro, H.P., Hermes-Lima Pereira, B., Demasi, M., Costa, D.S.P., Abdalla, J., Onuki, C.M.A., Wendel, P.D., and Masci, A. (1993). A free radical hypothesis of lead poisoning and in born porphyrias associated with 5-aminolevulinic acid overload. Quim. Nova 16, 385–392.

de Bem, A.F., Fiuza, B., Calcerrada, P., Brito, P.M., Peluffo, G., Dinis, T.C.P., Trujillo, M., Rocha, J.B.T., Radi, R., and Almeida, L.M. (2013). Protective effect of diphenyl diselenide against peroxynitrite-mediated endothelial cell death: a comparison with ebselen. Nitric Oxide Biol. Chem. 31, 20–30.

Berlin, M., Zalups, R.., and Fowler, B.A. (2007). Handbook on the Toxicology of Metals (Amsterdam, Holland: Elsevier).

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brandão, R., Santos, F.W., Farina, M., Zeni, G., Bohrer, D., Rocha, J.B.T., and Nogueira, C.W. (2006). Antioxidants and metallothionein levels in mercury-treated mice. Cell Biol. Toxicol. 22, 429–438.

Brandão, R., Borges, L.P., de Oliveira, R., Rocha, J.B.T., and Nogueira, C.W. (2008). Diphenyl diselenide protects against hematological and immunological alterations induced by mercury in mice. J. Biochem. Mol. Toxicol. 22, 311–319.

Brandão, R., Borges, L.P., and Nogueira, C.W. (2009). Concomitant administration of sodium 2,3-dimercapto-1-propanesulphonate (DMPS) and diphenyl diselenide reduces effectiveness of DMPS in restoring damage induced by mercuric chloride in mice. Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 47, 1771–1778.

Brandão, R., de Oliveira, R., and Nogueira, C.W. (2010). Association between diphenyl diselenide and cadmium chloride attenuates the toxicity of both in tissues of mice in vitro. Toxicol. Vitro Int. J. Publ. Assoc. BIBRA 24, 1736–1742.

Carocci, A., Rovito, N., Sinicropi, M.S., and Genchi, G. (2014). Mercury toxicity and neurodegenerative effects. Rev. Environ. Contam. Toxicol. 229, 1–18.

Clarkson, T.W. (1997). The toxicology of mercury. Crit. Rev. Clin. Lab. Sci. 34, 369–403.

Counter, S.A., and Buchanan, L.H. (2004). Mercury exposure in children: a review. Toxicol. Appl. Pharmacol. 198, 209–230.

Devlin, T.M. (1997). Texbook of biochemistry with clinical correlations (New York: Wiley).

Edelstein, C.L. (2008). Biomarkers of acute kidney injury. Adv. Chronic Kidney Dis. 15, 222–234.

Ellman, G.L. (1959). Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82, 70–77.

Emanuelli, T., Rocha, J.B., Pereira, M.E., Porciuncula, L.O., Morsch, V.M., Martins, A.F., and Souza, D.O. (1996). Effect of mercuric chloride intoxication and dimercaprol treatment on delta-aminolevulinate dehydratase from brain, liver and kidney of adult mice. Pharmacol. Toxicol. 79, 136–143.

Favero, A.M., Oliveira, C.S., Franciscato, C., Oliveira, V.A., Pereira, J.S.F., Bertoncheli, C.M., da Luz, S.C.A., Dressler, V.L., Flores, E.M.M., and Pereira, M.E. (2014). Lactating and nonlactating rats differ to renal toxicity induced by mercuric chloride: the preventive effect of zinc chloride. Cell Biochem. Funct. 32, 420–428.

Ferreira, L.M., Cervi, V.F., Sari, M.H.M., Barbieri, A.V., Ramos, A.P., Copetti, P.M., de Brum, G.F., Nascimento, K., Nadal, J.M., Farago, P.V., et al. (2018). Diphenyl diselenide loaded poly(ε-caprolactone) nanocapsules with selective antimelanoma activity: Development and cytotoxic evaluation. Mater. Sci. Eng. C Mater. Biol. Appl. 91, 1–9.

Fiuza, T. da L., Oliveira, C.S., da Costa, M., Oliveira, V.A., Zeni, G., and Pereira, M.E. (2015). Effectiveness of (PhSe)2 in protect against the HgCl2 toxicity. J. Trace Elem. Med. Biol. Organ Soc. Miner. Trace Elem. GMS 29, 255–262.

Flohe, L., Günzler, W.A., and Schock, H.H. (1973). Glutathione peroxidase: a selenoenzyme. FEBS Lett. 32, 132–134.

Franciscato, C., Moraes-Silva, L., Duarte, F.A., Oliveira, C.S., Ineu, R.P., Flores, E.M.M., Dressler, V.L., Peixoto, N.C., and Pereira, M.E. (2011). Delayed biochemical changes induced by mercury intoxication are prevented by zinc pre-exposure. Ecotoxicol. Environ. Saf. 74, 480–486.

Graeme, K.A., and Pollack, C.V. (1998). Heavy metal toxicity, Part I: arsenic and mercury. J. Emerg. Med. 16, 45–56.

Hazelhoff, M.H., Bulacio, R.P., Chevalier, A., and Torres, A.M. (2018). Renal expression of organic anion transporters is modified after mercuric chloride exposure: Gender-related differences. Toxicol. Lett. 295, 390–396.

Klaassen, C.D., Wonsiewics, M.J., and McCurdy, P. (1996). Pharmacological Basis of therapeutics (New York: McGraw-Hill).

Kuraś, R., Janasik, B., Wąsowicz, W., and Stanisławska, M. (2018). Revision of reciprocal action of mercury and selenium. Int. J. Occup. Med. Environ. Health 31, 575–592.

Leitemperger, J., Menezes, C., Santi, A., Murussi, C., Lópes, T., Costa, M., Nogueira, L.S., and Loro, V.L. (2016). Early biochemical biomarkers for zinc in silver catfish (Rhamdia quelen) after acute exposure. Fish Physiol. Biochem. 42, 1005–1014.

Li, P., Feng, X.B., Qiu, G.L., Shang, L.H., and Li, Z.G. (2009). Mercury pollution in Asia: a review of the contaminated sites. J. Hazard. Mater. 168, 591–601.

Lu, S.C. (2013). Glutathione synthesis. Biochim. Biophys. Acta 1830, 3143–3153.

da Luz Fiuza, T., Leitemperger, J., Severo, E.S., Marins, A.T., do Amaral, A.B., Pereira, M.E., and Loro, V.L. (2018). Effects of diphenyl diselenide diet on a model of mercury poisoning. Mol. Biol. Rep.

Meister, A. (1988). Glutathione metabolism and its selective modification. J. Biol. Chem. 263, 17205–17208.

Meotti, F.C., Stangherlin, E.C., Zeni, G., Nogueira, C.W., and Rocha, J.B.T. (2004). Protective role of aryl and alkyl diselenides on lipid peroxidation. Environ. Res. 94, 276–282.

Mesquita, M., Pedroso, T.F., Oliveira, C.S., Oliveira, V.A., do Santos, R.F., Bizzi, C.A., and Pereira, M.E. (2016). Effects of zinc against mercury toxicity in female rats 12 and 48 hours after HgCl2 exposure. EXCLI J. 15, 256–267.

Moraes-Silva, L., Bueno, T.M., Franciscato, C., Ineu, R.P., and Pereira, M.E. (2012a). Effectiveness of copper chloride in protecting against alterations induced by mercury chloride in newborn rats. J. Biochem. Mol. Toxicol. 26, 354–359.

Moraes-Silva, L., Bueno, T.M., Franciscato, C., de Oliveira, C.S., Peixoto, N.C., and Pereira, M.E. (2012b). Mercury chloride increases hepatic alanine aminotransferase and glucose 6-phosphatase activities in newborn rats in vivo. Cell Biol. Int. 36, 561–566.

Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.

Oliveira, V.A., Oliveira, C.S., Ineu, R.P., Moraes-Silva, L., de Siqueira, L.F., and Pereira, M.E. (2014). Lactating and non-lactating rats differ in sensitivity to HgCl(2): Protective effect of ZnCl(2). J. Trace Elem. Med. Biol. Organ Soc. Miner. Trace Elem. GMS 28, 240–246.

Oliveira, V.A., Oliveira, C.S., Mesquita, M., Pedroso, T.F., Costa, L.M., Fiuza, T. da L., and Pereira, M.E. (2015). Zinc and N-acetylcysteine modify mercury distribution and promote increase in hepatic metallothionein levels. J. Trace Elem. Med. Biol. Organ Soc. Miner. Trace Elem. GMS 32, 183–188.

Oskarsson, A., and Fowler, B.A. (1985). Effects of lead on the heme biosynthetic pathway in rat kidney. Exp. Mol. Pathol. 43, 409–417.

Peixoto, N.C., and Pereira, M.E. (2007). Effectiveness of ZnCl2 in protecting against nephrotoxicity induced by HgCl2 in newborn rats. Ecotoxicol. Environ. Saf. 66, 441–446.

Peixoto, N.C., Roza, T., Flores, E.M.M., and Pereira, M.E. (2003). Effects of zinc and cadmium on HgCl2-delta-ALA-D inhibition and Hg levels in tissues of suckling rats. Toxicol. Lett. 146, 17–25.

Peixoto, N.C., Serafim, M.A., Flores, E.M.M., Bebianno, M.J., and Pereira, M.E. (2007). Metallothionein, zinc, and mercury levels in tissues of young rats exposed to zinc and subsequently to mercury. Life Sci. 81, 1264–1271.

Ravel, R. (1997). Laboratório clínico: aplicações clínicas dos dados laboratoriais (Rio de Janeiro).

Rocha, J.B., Pereira, M.E., Emanuelli, T., Christofari, R.S., and Souza, D.O. (1995). Effect of treatment with mercury chloride and lead acetate during the second stage of rapid postnatal brain growth on delta-aminolevulinic acid dehydratase (ALA-D) activity in brain, liver, kidney and blood of suckling rats. Toxicology 100, 27–37.

Rooney, J.P.K. (2007). The role of thiols, dithiols, nutritional factors and interacting ligands in the toxicology of mercury. Toxicology 234, 145–156.

Sassa, S. (1982). Delta-aminolevulinic acid dehydratase assay. Enzyme 28, 133–145.

Sheehan, M.C., Burke, T.A., Navas-Acien, A., Breysse, P.N., McGready, J., and Fox, M.A. (2014). Global methylmercury exposure from seafood consumption and risk of developmental neurotoxicity: a systematic review. Bull. World Health Organ. 92, 254-269F.

Silvestre, F., Danielski, L.G., Michels, M., Florentino, D., Vieira, A., Souza, L., Cardoso, L.C., Schraiber, R., Rezin, G.T., Vuolo, F., et al. (2014). Effects of organoselenium compounds on early and late brain biochemical alterations in sepsis-survivor rats. Neurotox. Res. 26, 382–391.

Streets, D.G., Devane, M.K., Lu, Z., Bond, T.C., Sunderland, E.M., and Jacob, D.J. (2011). All-time releases of mercury to the atmosphere from human activities. Environ. Sci. Technol. 45, 10485–10491.

Thomas, L. (1998). Clinical laboratory diagnostics (Frankfurt).

Vedavathi, M., Girish, K.S., and Kumar, M.K. (2004). Isolation and characterization of cytosolic alanine aminotransferase isoforms from starved rat liver. Mol. Cell. Biochem. 267, 13–23.

WHO (2003). Inorganic mercury, Envionmental Health Criteria 118.

Zalups, R.K. (2000). Molecular interactions with mercury in the kidney. Pharmacol. Rev. 52, 113–143.

Paulmier, C. (1986). Seleno organic functional groups. (Oxford).

Subtitles

Fig. 1. Liver (A), kidney (B) and blood (C) δ-ALA-D activity. Male Swiss albino mice were treated during thirty consecutive days with (PhSe)2 supplemented feed (3.0 mg/kg/day) or commercial feed, and exposed to HgCl2 (5.0 mg/kg/day, s.c.) or saline in the last five days. The results are expressed as mean ± S.E.M. Duncan's multiple range test: groups statistically equals are accompanied of the same letters and groups statistically different are accompanied by different letters ($p \leq 0.05$; n = 6–8).

Fig. 2. Urea (A) and creatinine (B) levels from male Swiss albino mice treated as described in Fig. 1. The results are expressed as mean ± S.E.M. Duncan's multiple range test: groups statistically equals are accompanied by the same letters, and groups statistically different are accompanied by different letters ($p \leq 0.05$; n =6–8).

Fig. 3. AST (A) and ALT (B) activity from male Swiss albino mice treated as described in Fig. 1. The results are expressed as mean ± S.E.M. Duncan's multiple range test: groups statistically equals are accompanied by the same letters, and groups statistically different are accompanied by different letters ($p \leq 0.05$; n =6–8).

Fig. 4. Liver (A-C) total and non-protein thiols levels and kidney (B-D) total and non-protein thiols levels from Swiss albino mice treated as described in Fig. 1. The results are expressed as mean ± S.E.M. Duncan's multiple range test: groups statistically equals are accompanied by the same letters, and groups statistically different are accompanied by different letters ($p \leq 0.05$; n = 6–8).

Fig. 5. Liver (A), kidney (B) TBARS levels from male Swiss albino mice treated as described in Fig. 1. The results are expressed as mean ± S.E.M. Duncan's multiple range test: groups statistically equals are accompanied by the same letters, and groups statistically different are accompanied by different letters ($p \leq 0.05$; n =6–8).

Figures

Fig.1.

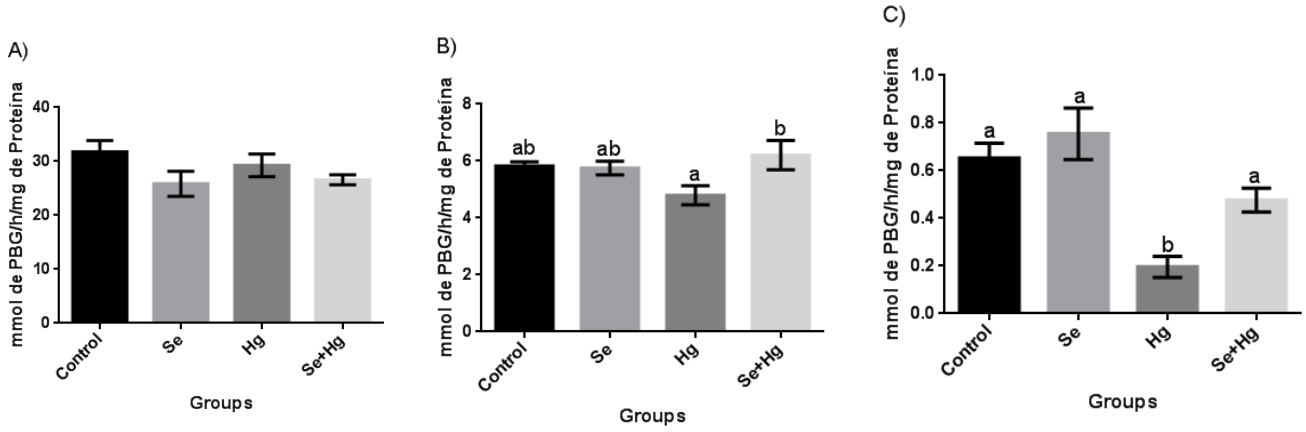


Fig. 2.

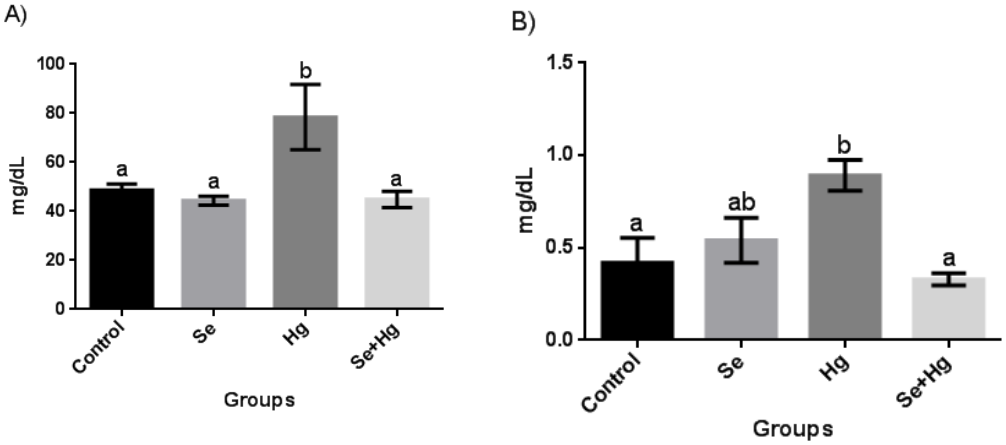


Fig. 3.

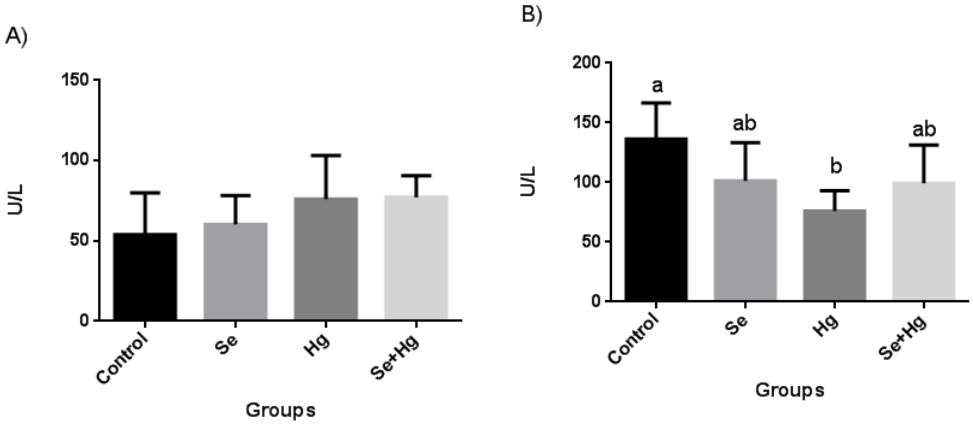
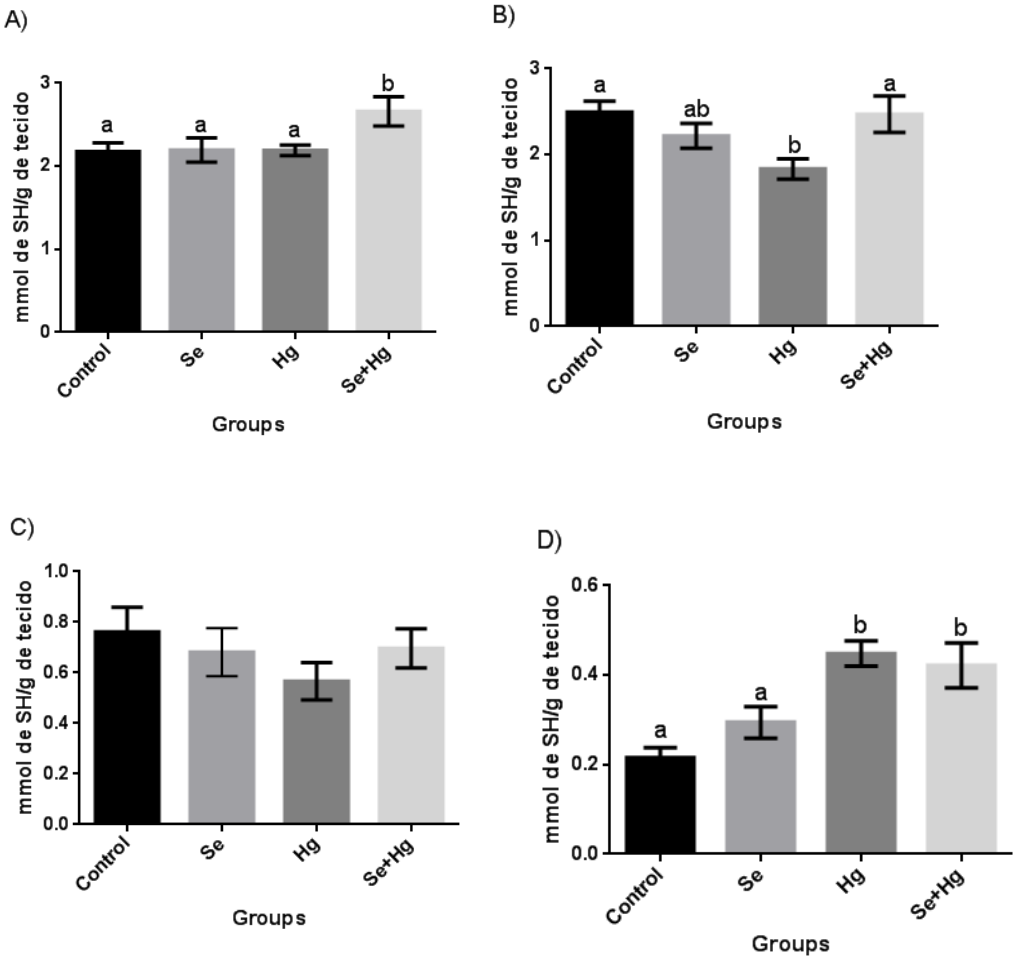


Fig. 4.


A)
mmol de SH/g de tecido
a
a
a
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Control
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B)
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ab
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Control
Se
Hg
Se+Hg
Groups
C)
mmol de SH/g de tecido
Control
Se
Hg
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Groups
D)
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a
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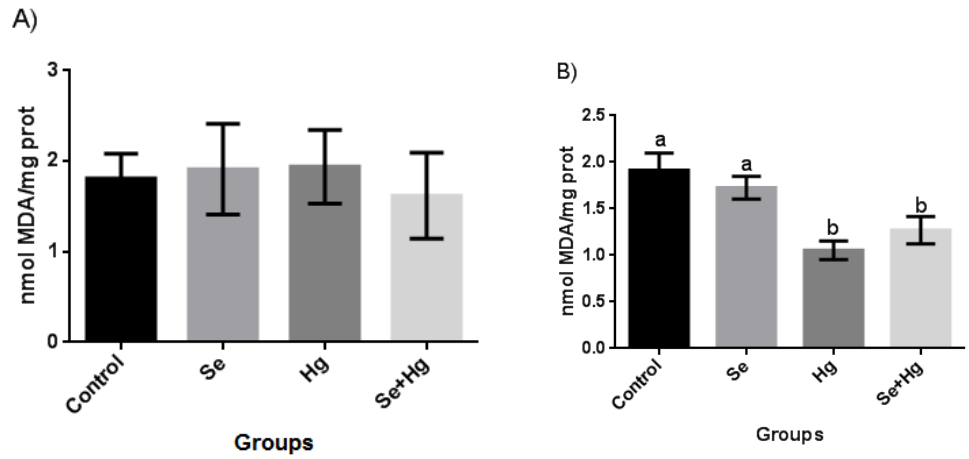
b
Control
Se
Hg
Se+Hg
Groups

Fig. 5


A)
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Groups

Table 1

Hg and Zn levels in liver, kidney and blood from male Swiss albino mice treated during thirty days with $(PhSe)_2$ supplemented feed (3.0 mg / kg) or control feed and exposed (s.c) to $HgCl_2$ (5.0 mg / kg) or Saline (0.9%) for 5 days.

Group	Liver	Kidney	Blood
Hg levels (µg Hg/g tissue)			
Control	0.213±0.00[a]	0.21±0.00[a]	nd
Se	0.215±0.00[a]	0.19±0.00[a]	nd
Hg	0.398±0.03[b]	1.906±0.14[b]	nd
Se-Hg	0.300±0.02[b]	1.517±0.20[b]	nd
Zn levels (µg Zn/g tissue)			
Control	2.437±0.11	1.454±0.01[a]	0.307±0.00
Se	2.225±0.11	1.354±0.01[a]	0.293±0.01
Hg	2.662±0.10	1.818±0.08[b]	0.258±0.00
Se-Hg	3.366±0.92	1.862±0.13[b]	0.301±0.02

Data are expressed as mean ± S.E.M. (n = 3) and the values accompanied by different letters in the same column are statistically different ($p \leq 0.05$). The sample whose mercury concentration were below the detectable limit of the technique are represented by nd (non-detected).

6. DISCUSSÃO

A utilização do mercúrio em diferentes setores faz com que o contato com seres humanos e outros organismos, seja frequente e dificilmente evitável (ZALUPS, 2000). Sabe-se que o mercúrio inorgânico sofre alquilação, processo que torna esse elemento mais lipossolúvel, facilitando, dessa forma, o seu transporte através das membranas celulares, e por consequência, fazendo com que a bioacumulação nos tecidos do organismo seja possível. Esse fenômeno ocorre com invertebrados, peixes e mamíferos, sendo também possível ocorrer em plantas aquáticas (WHO, 1991). Por ser um metal tóxico e exercer efeitos nocivos para diferentes organismos, é importante encontrar alternativas com o objetivo de combater ou pelo menos reduzir os efeitos tóxicos causados pelo mercúrio. Sendo assim, esse estudo buscou demonstrar a ação do mercúrio, quanto aos seus efeitos tóxicos, em peixes e mamíferos, duas espécies diferentes, mas com igual importância ao se avaliar os impactos do metal no meio ambiente. Além disso, essa pesquisa teve como propósito buscar alternativas de prevenção contra a intoxicação por Hg, utilizando um composto com selênio, que é um micronutriente essencial para o desenvolvimento e também para as defesas do organismo.

Este estudo demonstrou que a exposição ao cloreto de mercúrio causou alterações na atividade da enzima δ-ALA-D de todos os animais expostos e em ambos os modelos experimentais testados. Durante 5 dias consecutivos recebendo uma dose diária de cloreto de mercúrio (1,7 mg/kg de $HgCl_2$) os jundiás (**artigo**) apresentaram uma inibição na atividade da enzima δ-ALA-D nos tecidos sanguíneos e renal (aproximadamente 40% em ambos os tecidos). No experimento utilizando os camundongos a exposição diária (5,0mg/kg de $HgCl_2$) durante 5 dias, provocou uma inibição estatisticamente significativa (aproximadamente 70%) apenas na δ-ALA-D sanguínea (**manuscrito**). Contudo, pode-se observar uma pequena redução na atividade da δ-ALA-D renal dos camundongos expostos ao Hg (aproximadamente 18%) (**manuscrito**). Essa enzima é de grande importância para o metabolismo aeróbico, uma vez que ela possui a função de catalisar a condensação de duas moléculas do ácido δ-aminolevulínico para formar o composto monopirrólico porfobilinogênio, sendo este o produto do segundo passo para a biossíntese do heme. Além da sua importância para o metabolismo, a enzima δ-ALA-D vem sendo utilizada como um marcador de exposição a produtos tóxicos tanto em animais em laboratório (FIUZA et al., 2015; LEITEMPERGER et al., 2016; MENEZES et al., 2016; MESQUITA et al., 2016; OLIVEIRA et al., 2015; PEIXOTO et al., 2007) como em animais selvagens para monitoramento de contaminação ambiental (COMPANY et al., 2011; ESPÍN et al., 2015). Por esse motivo, elegemos a enzima δ-ALA-D como marcador de efeito tóxico, e como demonstrado nos resultados de ambos os experimentos, os animais expostos ao Hg apresentaram inibição da δ-ALA-D em pelo menos um

dos tecidos avaliados. A ausência de efeitos sobre a atividade da δ-ALA-D hepática, tanto em peixes como em camundongos, se dá devido à forma inorgânica do Hg utilizada para o estudo, a qual possui como alvo principal o tecido renal.

O mecanismo pelo qual o Hg é transportado pelo organismo e conduzido até o tecido renal envolve uma característica do Hg^{2+}, que é a sua afinidade por grupamentos sulfidrílicos (-SH) (BERLIN; ZALUPS; *FOWLER*, 2007; LI et al., 2009; MAGOS; CLARKSON, 2006; ROCHA et al., 1995; ROONEY, 2007). Zalups (2000) afirma ainda que a grande afinidade entre esses dois compostos possibilita a formação de complexos de Hg com a cisteína (Cys) (aminoácido que contém SH em sua estrutura), que resultam em uma molécula chamada dicisteinilmercúrio (Cys-Hg-Cys). A membrana citoplasmática dos túbulos proximais renais possui um sistema de transporte que envolve a absorção da cisteína. Dessa forma, o mercúrio na forma de dicisteinilmercúrio consegue chegar até o interior das células dos túbulos proximais, onde pode se tornar novamente Hg^{2+} e encontrar a enzima δ-ALA-D, fazendo com que ocorra uma inibição de sua atividade catalítica.

Conforme observado no experimento com peixes, podemos observar que os animais que foram expostos ao Hg apresentaram uma diminuição nos níveis de TBARS, tióis totais e tióis não proteicos no rim (**artigo**). Quando analisados os rins dos camundongos expostos ao Hg, estes apresentaram uma diminuição nos níveis de TBARS e tióis totais. Diferentemente dos resultados obtidos no artigo, o tecido renal dos camundongos que receberam mercúrio apresentou um aumento nos níveis de tiois não proteicos (**manuscrito**). Sabe-se que a exposição ao mercúrio pode induzir ao dano oxidativo nas membranas celulares dos diferentes tecidos, podendo ser observado pela formação de espécies reativas ao ácido tiobarbitúrico (TBARS). Isso ocorre devido à formação de espécies reativas de oxigênio (ROS) e esses eventos conduzem a um aumento nos níveis de TBARS em vários tecidos, o que pode levar a um distúrbio na função fisiológica normal de peixes e mamíferos (FUKINO et al., 1984; HUSSAIN et al., 1999). A diminuição nos níveis de TBARS pode ser explicada pelo fato de que foram utilizadas doses de $HgCl_2$ que são sub letais, 1,7mg/kg (**artigo**) e 5,0 mg/kg (**manuscrito**). O mesmo já foi observado em outros trabalhos realizados pelo nosso grupo de pesquisa, e acreditamos que essa diminuição nos níveis de TBARS são devido à exposição do organismo a doses baixas de xenobióticos, no entanto, suficientes para causar alterações nos sistemas de defesa, o que representa uma resposta adaptativa do organismo devido a exposição ao agente toxico (FIUZA et al., 2015; LEITEMPERGER et al., 2016).

A nefrotoxicidade do mercúrio se manifesta principalmente por falência renal aguda em seres humanos e animais experimentais, caracterizada por necrose do epitélio renal,

principalmente dos túbulos proximais, acompanhado de glomérulo nefrite e proteinúria (CLARKSON, 1997; GOYER R.; KLAASSEN; WALLKES M.P, 1995; ZALUPS, 2000). A diminuição nos níveis de tiois totais observada em ambos os experimentos pode ter ocorrido devido aos danos na estrutura e na função renal, o que conduz ao aumento da eliminação de proteínas na urina. O aumento da excreção de proteínas e o aumento da atividade de algumas enzimas na urina podem ser indicativos de tubulopatia na disfunção renal em indivíduos expostos ao Hg (BARREGÅRD et al., 1988; KOBAL et al., 2000). Quando avaliados os níveis de tiois não proteicos de rim, houve uma diferença entre os resultados obtidos entre os dois experimentos. Enquanto no experimento realizado com peixes os níveis de TNP reduziram (**artigo**), no experimento com camundongos os níveis de TNP aumentaram (**manuscrito**). O tripeptideo glutationa (GSH) representa o TNP endógeno mais abundante presente em todos tecidos e desempenha um papel importante na proteção de células (ROONEY, 2007). Resultados controversos têm sido obtidos com relação aos efeitos do mercúrio nos níveis de GSH. Zalups (2000) demonstrou que baixas doses de mercúrio podem aumentar os níveis de GSH renal, enquanto que altas doses deste metal podem reduzir os níveis de GSH renal. Acredita-se que essa diferença entre resultados, observada nos experimentos, ocorra devido à via de administração do $HgCl_2$ utilizada em cada um deles, visto que nos peixes o Hg foi administrado via intraperitoneal (**artigo**), considerada uma via com mais rápida de absorção e consequentemente uma quantidade maior de mercúrio entra em contato com os diferentes tecidos, enquanto a via sub cutânea (**manuscrito**), utilizada para administração de Hg nos camundongos, é uma via cuja absorção do Hg ocorre de forma mais lenta, com isso o organismo tem um tempo maior para criar mecanismos para proteger os órgãos dos efeitos tóxicos do Hg.

Devido ao seu grande fluxo sanguíneo (25% do débito cardíaco) e intensa atividade enzimática na execução de suas funções, além de desempenhar importante papel na excreção de xenobióticos, o rim é o órgão-alvo preferencial da maioria destas substâncias (PASSOW; ROTHSTEIN; CLARKSON, 1961; VAN VLEET; SCHNELLMANN, 2003; ZALUPS, 1993). No presente estudo foi observada nefrotoxicidade causada pela exposição ao Hg em ambas as espécies analisadas. O dano ao tecido renal foi confirmado devido ao aumento nos níveis de creatinina sérica dos peixes expostos ao Hg, contudo os níveis de amônia não foram alterados (**artigo**). Como observado no experimento utilizando camundongos, os níveis de ureia e creatinina apresentaram-se elevados (**manuscrito**). O aumento dos níveis de ureia/amônia e creatinina é um efeito comumente associado à exposição ao mercúrio inorgânico devido a sua ação nefrotóxica (ZALUPS; LASH,

1994). A ureia é produto do catabolismo de proteínas, sendo a principal forma pela qual os mamíferos excretam o excesso de nitrogênio, no entanto a creatinina é o produto da degradação da fosfocreatina (BAUM; DICHOSO; CARLTON, 1975; CHAMPE; HARVEY; FERRIER, 2006). Assim como nos mamíferos, os peixes também excretam creatinina, contudo a principal forma de excreção de conteúdo nitrogenado é através da excreção de amônia, o que ocorre em torno de 50 a 84% (ALTINOK; GRIZZLE, 2004; KAJIMURA et al., 2004; LAM; JUSOH; LAW, 2008). Quando os rins se encontram saudáveis, estes metabólitos são transportados para o órgão e excretados através da urina. O aumento do nível sérico destes metabólitos é um indicativo de dano renal severo (BAUM; DICHOSO; CARLTON, 1975). Sendo assim, é evidente que a exposição ao $HgCl_2$ induz danos ao tecido renal em ambos os modelos experimentais estudados.

No presente estudo, foi observado um aumento nos níveis de mercúrio no fígado e no rim dos animais expostos ao $HgCl_2$ em ambos os experimentos realizados. Os resultados obtidos no experimento com peixes (**artigo**) e com camundongos (**manuscrito**) apresentaram concordância do Hg apresentar uma acumulação maior nos rins que no fígado (aproximadamente 2X e 5X respectivamente). Com poucas horas após a exposição, o mercúrio inorgânico acumula-se rapidamente no tecido renal, chegando até 50% de uma dose não tóxica (ZALUPS, 1993). O Hg tende a se acumular nas células epiteliais do túbulo proximal, no entanto, alguns efeitos da exposição ao Hg podem ser notados nos demais segmentos tubulares (ZALUPS, 2000). Os mecanismos pelo qual o Hg atravessa a membrana citoplasmática, acumulando-se nos túbulos proximais, ocorrem principalmente com a participação de transportadores de aminoácidos, como por exemplo a cisteína, dessa forma, o Hg na forma de dicisteinilmercúrio tem o seu transporte para o interior dos rins facilitado pelos mesmos transportadores. Com isso, desencadeia a toxicidade desse metal no sistema renal.

Os níveis de zinco (Zn), um elemento essencial requerido para o crescimento e desenvolvimento (KREBS, 1999), também foram medidos no sangue, nos rins e no fígado dos peixes e camundongos. De acordo com nossos dados, a exposição ao Hg causou alterações na homeostase do Zn no tecido hepático e sanguíneo dos peixes (**artigo**) enquanto nos camundongos a exposição ao Hg alterou apenas a homeostase renal do Zn (**manuscrito**). Mais uma vez, acredita-se que a diferença entre os resultados esteja relacionada com a via de administração do Hg utilizada nos experimentos. Uma vez que por via subcutânea (**manuscrito**) o Hg é absorvido mais lentamente, em menor quantidade e em tempo suficiente para conjugar os grupamentos -SH, posteriormente eliminados pelos rins, os efeitos tóxicos desse metal reduziriam nos tecidos hepáticos e sanguíneo. Embora tenham sido detectados níveis de Hg no tecido hepático, em ambos

os experimentos, os parâmetros de toxicidade não se mostraram alterados, com exceção da homeostase do Zn no fígado observada nos peixes (**artigo**). Além disso, o único parâmetro relacionado à toxicidade hepática, após a exposição ao Hg, foi a inibição na atividade da enzima alanina aminotransferase (ALT) em camundongos (**manuscrito**). Contudo, o efeito do Hg apresentado nesta enzima não caracteriza dano hepático, mas uma interação entre o Hg e os grupamentos –SH presentes no sitio ativo na ALT (VEDAVATHI; GIRISH; KUMAR, 2004).

Nos últimos anos, estudos foram realizados para compreender os mecanismos envolvidos na toxicidade do Hg e seus efeitos sobre as defesas endógenas do organismo. A exposição ao Hg pode causar uma diminuição no sistema endógeno de defesa antioxidante, inibindo a atividade das enzimas superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GPx) e glutationa redutase (GR), em tecido hepático, renal e cerebral de ratos (AGARWAL et al., 2010). Além do sistema antioxidante enzimático, diversas substâncias, tais como as vitaminas C e E, flavonoides e o tripeptídeo glutationa (GSH), representam o grupo não enzimático de substâncias com atividade antioxidante (REISCHL et al., 2007). As defesas endógenas do organismo também exercem ação contra os ataques que as células estão expostas frequentemente. Nos últimos anos vários estudos testaram substâncias que pudessem proteger e ampliar a longevidade celular. Neste sentido, este estudo demonstra o efeito do $(PhSe)_2$, administrado na alimentação dos animais (3,0 mg/kg de ração) contra os efeitos do Hg. A ração suplementada com $(PhSe)_2$ *per se*, não causou alteração significativa nos parâmetros analisados em ambos os modelos animais testado. No entanto, quando analisada a ação do $(PhSe)_2$ nos peixes sobre os efeitos do Hg, esta se mostrou benéfica, mesmo que apresentando proteção parcial sobre a elevação dos níveis de creatinina, diminuição dos níveis renais de TBARS e tióis não proteicos, além da homeostase sanguínea do Zn. Porém, a atividade da δ-ALA-D foi protegida totalmente pelo tratamento com $(PhSe)_2$ (**artigo**). Com base nesses resultados, acreditamos que a alimentação de 30 dias consecutivos com $(PhSe)_2$ possa ser efetiva para proteger contra a nefrotoxicidade induzida pelo Hg, uma vez que que os níveis de creatinina dos animais expostos ao Hg e alimentados com $(PhSe)_2$ foram parcialmente restabelecidos, bem como pela proteção contra a inibição da enzima δ-ALA-D.

Assim como no modelo utilizando peixes, os camundongos também apresentaram resultados que indicam que o $(PhSe)_2$ na dieta pode ser uma alternativa promissora contra os efeitos tóxicos do Hg, com ênfase nos efeitos sobre o tecido renal. Além disso o $(PhSe)_2$ protegeu totalmente a inibição da δ-ALA-D renal e sanguínea, a elevação dos níveis de ureia e creatinina e diminuição dos níveis de tiois totais de rim, do mesmo modo que protegeu parcialmente a inibição da ALT sérica. Porém, a redistribuição do Hg entre os tecidos e a homeostase do Zn não foi alterada pela dieta com

$(PhSe)_2$ (**manuscrito**). Com base nesses resultados, concluímos que os camundongos expostos ao $HgCl_2$ e alimentados com $(PhSe)_2$ apresentam uma condição renal melhor que os que receberam apenas o $HgCl_2$. Contudo, os níveis dos marcadores de toxicidade renal apresentaram-se iguais estatisticamente aos do controle, além dos níveis de tiois totais renais que foram mantidos, o que representa uma menor perda do conteúdo proteico do órgão. Acreditamos que o mecanismo envolvido na proteção do $(PhSe)_2$ esteja relacionado com a capacidade do composto aumentar os sistemas de defesa antioxidante, como por exemplo, o aumento da síntese de GSH (de BEM et al., 2013), e síntese de metalotioneinas (MT) (BRANDÃO et al., 2006), moléculas das quais que contem grupamentos –SH e possuem atividade "scavenger" no organismo. Devido à afinidade do Hg por grupos SH, supõe-se uma interação entre essas moléculas e o Hg, de forma que se forme um complexo inerte (GSH/MT-Hg), impedindo a interação do Hg com outras biomoléculas, até que o Hg possa ser eliminado.

6. CONCLUSÕES

A partir dos resultados desse estudo, podemos concluir que:

1. Peixes e camundongos são sensíveis aos efeitos tóxicos do Hg em ambos os modelos experimentais testados:
    - Em ambos os experimentos, os animais apresentaram evidências clássicas da intoxicação pela forma inorgânica do Hg, sendo que o tecido renal foi o mais afetado.
    - Peixes e camundongos respondem de maneira semelhante no que diz respeito à acumulação e distribuição do Hg entre os tecidos, conforme observado com o maior acúmulo de Hg no tecido renal.
    - A via de administração do Hg pode ter sido responsável pela discordância entre alguns resultados, uma vez que a velocidade de absorção Hg mostra ser diferente entre as vias, sendo, portanto, um fator a ser considerado quando se determina um modelo experimental.
    - Não foi evidenciado dano hepático causado pela exposição ao $HgCl_2$ em ambos os modelos experimentais.
    - A enzima δ-ALA-D pode ser usada como marcador de exposição ao Hg.
2. A alimentação com $(PhSe)_2$ pode ser uma alternativa promissora para prevenir os efeitos da exposição ao Hg, tendo em vista que:
    - Houve uma melhora nos níveis dos marcadores de toxicidade renal, bem como uma proteção contra a inibição da enzima δ-ALA-D dos tecidos renal e sanguíneo.

REFERÊNCIAS

AGARWAL, R. et al. Role of vitamin E in preventing acute mercury toxicity in rat. **Environmental Toxicology and Pharmacology**, v. 29, n. 1, p. 70–78, jan. 2010.

ALTINOK, I.; GRIZZLE, J. M. Excretion of ammonia and urea by phylogenetically diverse fish species in low salinities. v. 238, p. 499–507, 2004.

ANDRADE, A.; PINTO, S. .; OLIVEIRA, R. . **Animais de Laboratório: criação e experimentação**. Rio de Janeiro: FIOCRUZ, 2002.

ANTOINE, D. J. et al. Mechanism-based bioanalysis and biomarkers for hepatic chemical stress. **Xenobiotica; the Fate of Foreign Compounds in Biological Systems**, v. 39, n. 8, p. 565–577, ago. 2009.

AU, D. W. T. The application of histo-cytopathological biomarkers in marine pollution monitoring: a review. **Marine Pollution Bulletin**, v. 48, n. 9–10, p. 817–834, maio 2004.

AZEVEDO, F. A.; NASCIMENTO, E. S.; CHASIN, A. Aspectos Atualizados dos Riscos Toxicológicos do Mercúrio. v. 16, n. 3, p. 87–104, 2001.

BARBOSA, N. B. V. et al. Diphenyl diselenide reduces temporarily hyperglycemia: possible relationship with oxidative stress. **Chemico-Biological Interactions**, v. 163, n. 3, p. 230–238, 7 nov. 2006.

BARBOSA, N. B. V. et al. Dietary diphenyl diselenide reduces the STZ-induced toxicity. **Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association**, v. 46, n. 1, p. 186–194, jan. 2008.

BARCELLOS, L. J. G.; KREUTZ, L. C.; QUEVEDO, R. M. Nursery rearing of jundiá, Rhamdia quelen (Quoy & Gaimard) in cages: cage type, stocking density and stress response to confinement. v. 232, p. 383–394, 2004.

BARREGÅRD, L. et al. Enzymuria in workers exposed to inorganic mercury. **International Archives of Occupational and Environmental Health**, v. 61, n. 1–2, p. 65–69, 1988.

BAUM, N.; DICHOSO, C. C.; CARLTON, C. E. Blood urea nitrogen and serum creatinine. Physiology and interpretations. **Urology**, v. 5, n. 5, p. 583–588, maio 1975.

BECHARA, E. J. Oxidative stress in acute intermittent porphyria and lead poisoning may be triggered by 5-aminolevulinic acid. **Brazilian Journal of Medical and Biological Research = Revista Brasileira De Pesquisas Medicas E Biologicas**, v. 29, n. 7, p. 841–851, jul. 1996.

BERLIN, M.; ZALUPS, R. .; FOWLER, B. A. **Handbook on the Toxicology of Metals**. Third ed. Amsterdam, Holland: Elsevier, 2007.

BERNARD, A.; LAUWERYS, R. Metal-induced alterations of delta-aminolevulinic acid dehydratase. **Annals of the New York Academy of Sciences**, v. 514, p. 41–47, 1987.

BERZAS NEVADO, J. J. et al. Mercury in the Tapajós River basin, Brazilian Amazon: a review. **Environment International**, v. 36, n. 6, p. 593–608, ago. 2010.

BISINOTI, M. C.; JARDIM, W. F. Behavior of methylmercury in the environment. v. 27, p. 593–600, 2004.

BORGES, L. P. et al. Protective effect of diphenyl diselenide on acute liver damage induced by 2-nitropropane in rats. **Toxicology**, v. 210, n. 1, p. 1–8, 15 maio 2005.

BORGES, L. P. et al. Acute liver damage induced by 2-nitropropane in rats: effect of diphenyl diselenide on antioxidant defenses. **Chemico-Biological Interactions**, v. 160, n. 2, p. 99–107, 25 mar. 2006.

BRANDÃO, R. et al. Antioxidants and metallothionein levels in mercury-treated mice. **Cell Biology and Toxicology**, v. 22, n. 6, p. 429–438, nov. 2006.

CARNEIRO, P. C. F. **A produção do jundiá em cativeiro**. Santa Maria: UFSM, 2004.

CARNEIRO, P. C. F.; MIKOS, J. D. Frequência alimentar de alevinos de jundiá, Rhamdia quelen. v. 35, p. 187–191, 2005.

CASTILHOS, Z. C. et al. Mercury contamination in fish from gold mining areas in Indonesia and human health risk assessment. **The Science of the Total Environment**, v. 368, n. 1, p. 320–325, 1 set. 2006.

CHAMPE, P. .; HARVEY, R. .; FERRIER, D. . **Bioquimica Ilustrada**. 3º ed. Porto Alegre: Artmed, 2006.

CHAN, H. M. et al. Impacts of mercury on freshwater fish-eating wildlife and humans. v. 9, p. 867–883, 2003.

CLARKSON, T. W. The toxicology of mercury. **Critical Reviews in Clinical Laboratory Sciences**, v. 34, n. 4, p. 369–403, 1997.

COMPANY, R. et al. Source and impact of lead contamination on δ-aminolevulinic acid dehydratase activity in several marine bivalve species along the Gulf of Cadiz. **Aquatic Toxicology (Amsterdam, Netherlands)**, v. 101, n. 1, p. 146–154, 17 jan. 2011.

COUNTER, S. A.; BUCHANAN, L. H. Mercury exposure in children: a review. **Toxicology and Applied Pharmacology**, v. 198, n. 2, p. 209–230, 15 jul. 2004.

DA LUZ FIUZA, T. et al. Effects of diphenyl diselenide diet on a model of mercury poisoning. **Molecular Biology Reports**, 23 out. 2018.

DUMONT, E.; VANHAECKE, F.; CORNELIS, R. Selenium speciation from food source to metabolites: a critical review. **Analytical and Bioanalytical Chemistry**, v. 385, n. 7, p. 1304–1323, ago. 2006.

EDELSTEIN, C. L. Biomarkers of acute kidney injury. **Advances in Chronic Kidney Disease**, v. 15, n. 3, p. 222–234, jul. 2008.

ESPÍN, S. et al. Delta-aminolevulinic acid dehydratase (δALAD) activity in four free-living bird species exposed to different levels of lead under natural conditions. **Environmental Research**, v. 137, p. 185–198, fev. 2015.

FARINA, M. et al. Profile of nonprotein thiols, lipid peroxidation and delta-aminolevulinate dehydratase activity in mouse kidney and liver in response to acute exposure to mercuric chloride and sodium selenite. **Toxicology**, v. 184, n. 2–3, p. 179–187, 3 mar. 2003.

FARINA, M.; ASCHNER, M.; ROCHA, J. B. T. Oxidative stress in MeHg-induced neurotoxicity. **Toxicology and Applied Pharmacology**, v. 256, n. 3, p. 405–417, 1 nov. 2011.

FIUZA, T. DA L. et al. Effectiveness of (PhSe)2 in protect against the HgCl2 toxicity. **Journal of trace elements in medicine and biology: organ of the Society for Minerals and Trace Elements (GMS)**, v. 29, p. 255–262, jan. 2015.

FOWLER, B. A. Mechanisms of kidney cell injury from metals. **Environmental Health Perspectives**, v. 100, p. 57–63, abr. 1993.

FRANCISCATO, C. et al. Delayed biochemical changes induced by mercury intoxication are prevented by zinc pre-exposure. **Ecotoxicology and Environmental Safety**, v. 74, n. 3, p. 480–486, mar. 2011.

FUKINO, H. et al. Effect of zinc pretreatment on mercuric chloride-induced lipid peroxidation in the rat kidney. **Toxicology and Applied Pharmacology**, v. 73, n. 3, p. 395–401, maio 1984.

GBEM, T. T. et al. Trace metal accumulation in Clarias gariepinus (Teugels) exposed to sublethal levels of tannery effluent. **The Science of the Total Environment**, v. 271, n. 1–3, p. 1–9, 23 abr. 2001.

GHISLENI, G. et al. Diphenyl diselenide protects rat hippocampal slices submitted to oxygen-glucose deprivation and diminishes inducible nitric oxide synthase immunocontent. **Brain Research**, v. 986, n. 1–2, p. 196–199, 3 out. 2003.

GODWIN, H. A. The biological chemistry of lead. **Current Opinion in Chemical Biology**, v. 5, n. 2, p. 223–227, abr. 2001.

GOYER R.; KLAASSEN, C. D.; WALLKES M.P. Metal toxicology. p. 35–37, 1995.

GRAEME, K. A.; POLLACK, C. V. Heavy metal toxicity, Part I: arsenic and mercury. **The Journal of Emergency Medicine**, v. 16, n. 1, p. 45–56, fev. 1998.

GUILHERME, L. R. G. et al. Elementos-traço em solos e sistemas aquáticos. v. 4, p. 345–390, 2005.

GUNNAR, F. N. et al. **Handbook on the Toxicology of Metals**. 3º ed. Sandstead: Au, W., 2007.

HAMILTON, S. J. Review of selenium toxicity in the aquatic food chain. **The Science of the Total Environment**, v. 326, n. 1–3, p. 1–31, 29 jun. 2004.

HANSDA, A.; KUMAR, V.; USMANI, Z. Phytoremediation of heavy metals contaminated soil using plant growth promoting rhizobacteria (PGPR): a current perspective. p. 131–134, 2014.

HEATH, A. G. **Water pollution and fish physiology**. Virginia: Lewis Publishers, 1995.

HUSSAIN, S. et al. Accumulation of mercury and its effect on antioxidant enzymes in brain, liver, and kidneys of mice. **Journal of Environmental Science and Health. Part. B, Pesticides, Food Contaminants, and Agricultural Wastes**, v. 34, n. 4, p. 645–660, jul. 1999.

JAFFE, E. K. Porphobilinogen synthase, the first source of heme's asymmetry. **Journal of Bioenergetics and Biomembranes**, v. 27, n. 2, p. 169–179, abr. 1995.

KADAR, I.; KONCZ, J.; FEKETE, S. Experimental study of Cd, Hg, Mo, Pb and Se movement in soil-plant-animal system. p. 72–76, 2000.

KAJIMURA, M. et al. Dogmas and controversies in the handling of nitrogenous wastes: the effect of feeding and fasting on the excretion of ammonia, urea and other nitrogenous waste products in rainbow trout. **The Journal of Experimental Biology**, v. 207, n. Pt 12, p. 1993–2002, maio 2004.

KOBAL, A. B. et al. Renal function in miners intermittently exposed to elemental mercury vapour. **Arhiv Za Higijenu Rada I Toksikologiju**, v. 51, n. 4, p. 369–380, dez. 2000.

KREBS, N. F. Zinc transfer to the breastfed infant. **Journal of Mammary Gland Biology and Neoplasia**, v. 4, n. 3, p. 259–268, jul. 1999.

LA COLLA, N. S.; BOTTÉ, S. E.; MARCOVECCHIO, J. E. Mercury cycling and bioaccumulation in a changing coastal system: From water to aquatic organisms. **Marine Pollution Bulletin**, v. 140, p. 40–50, mar. 2019.

LACAZ, F. A. C.; PORTO, M. F. S.; PINHEIRO, T. M. M. Tragédias brasileiras contemporâneas: o caso do rompimento da barragem de rejeitos de Fundão/Samarco. p. 1–12, 2016.

LAM, S. S.; JUSOH, A.; LAW, A. T. Waste excretion of marble goby (Oxyeleotris marmorata Bleeker) fed with different diets. v. 274, p. 49–56, 2008.

LEBEL, J. et al. Evidence of early nervous system dysfunction in Amazonian populations exposed to low-levels of methylmercury. **Neurotoxicology**, v. 17, n. 1, p. 157–167, 1996.

LEITEMPERGER, J. et al. Early biochemical biomarkers for zinc in silver catfish (Rhamdia quelen) after acute exposure. **Fish Physiology and Biochemistry**, v. 42, n. 3, p. 1005–1014, jun. 2016.

LI, P. et al. Mercury pollution in Asia: a review of the contaminated sites. **Journal of Hazardous Materials**, v. 168, n. 2–3, p. 591–601, 15 set. 2009.

MAGALHÃES, L. E. A ciência e os animais de laboratório. v. 1, n. 1º, p. 7–13, 2012.

MAGOS, L.; CLARKSON, T. W. Overview of the clinical toxicity of mercury. **Annals of Clinical Biochemistry**, v. 43, n. Pt 4, p. 257–268, jul. 2006.

MARKHAM, G. D. et al. Spatial proximity and sequence localization of the reactive sulfhydryls of porphobilinogen synthase. **Protein Science: A Publication of the Protein Society**, v. 2, n. 1, p. 71–79, jan. 1993.

MENEZES, C. et al. Effect of diphenyl diselenide diet supplementation on oxidative stress biomarkers in two species of freshwater fish exposed to the insecticide fipronil. **Fish Physiology and Biochemistry**, v. 42, n. 5, p. 1357–1368, out. 2016.

MESQUITA, M. et al. Effects of zinc against mercury toxicity in female rats 12 and 48 hours after HgCl2 exposure. **EXCLI journal**, v. 15, p. 256–267, 2016.

MORAES-SILVA, L. et al. Effectiveness of copper chloride in protecting against alterations induced by mercury chloride in newborn rats. **Journal of Biochemical and Molecular Toxicology**, v. 26, n. 9, p. 354–359, set. 2012.

NOGUEIRA, C. W.; ROCHA, J. B. Diphenyl diselenide a Janus-Faced molecule. v. 21, p. 2055–2071, 2010.

NOGUEIRA, C. W.; ZENI, G.; ROCHA, J. B. T. Organoselenium and organotellurium compounds: toxicology and pharmacology. **Chemical Reviews**, v. 104, n. 12, p. 6255–6285, dez. 2004.

OLIVEIRA, V. A. et al. Lactating and non-lactating rats differ in sensitivity to HgCl(2): Protective effect of ZnCl(2). **Journal of trace elements in medicine and biology: organ of the Society for Minerals and Trace Elements (GMS)**, v. 28, n. 2, p. 240–246, abr. 2014.

OLIVEIRA, V. A. et al. Zinc and N-acetylcysteine modify mercury distribution and promote increase in hepatic metallothionein levels. **Journal of trace elements in medicine and biology: organ of the Society for Minerals and Trace Elements (GMS)**, v. 32, p. 183–188, out. 2015.

OZER, J. et al. The current state of serum biomarkers of hepatotoxicity. **Toxicology**, v. 245, n. 3, p. 194–205, 20 mar. 2008.

PALHETA, D.; TAYLOR, A. Mercury in environmental and biological samples from a gold mining area in the Amazon region of Brazil. **The Science of the Total Environment**, v. 168, n. 1, p. 63–69, 19 maio 1995.

PAPP, L. V. et al. From selenium to selenoproteins: synthesis, identity, and their role in human health. **Antioxidants & Redox Signaling**, v. 9, n. 7, p. 775–806, jul. 2007.

PARNHAM, M. J.; GRAF, E. Pharmacology of synthetic organic selenium compounds. **Progress in Drug Research. Fortschritte Der Arzneimittelforschung. Progres Des Recherches Pharmaceutiques**, v. 36, p. 9–47, 1991.

PASSOW, H.; ROTHSTEIN, A.; CLARKSON, T. W. The general pharmacology of the heavy metals. **Pharmacological Reviews**, v. 13, p. 185–224, jun. 1961.

PEIXOTO, N. C. et al. Metallothionein, zinc, and mercury levels in tissues of young rats exposed to zinc and subsequently to mercury. **Life Sciences**, v. 81, n. 16, p. 1264–1271, 29 set. 2007.

PEIXOTO, N. C.; PEREIRA, M. E. Effectiveness of ZnCl2 in protecting against nephrotoxicity induced by HgCl2 in newborn rats. **Ecotoxicology and Environmental Safety**, v. 66, n. 3, p. 441–446, mar. 2007.

PEROTTONI, J. et al. Renal and hepatic ALA-D activity and selected oxidative stress parameters of rats exposed to inorganic mercury and organoselenium compounds. **Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association**, v. 42, n. 1, p. 17–28, jan. 2004.

PETRAGNANI, N.; RODRIGUES, R.; COMASSETO, J. V. Organomet. p. 114–281, 1976.

PIRRONE, N. et al. Global Mercury emissions to the atmosphere from anthropogenic and natural sources. v. 10, p. 5951–5964, 2010.

PRATUSH, A.; KUMAR, A.; HU, Z. Adverse effect of heavy metals (As, Pb, Hg, and Cr) on health and their bioremediation strategies: a review. **International Microbiology: The Official Journal of the Spanish Society for Microbiology**, v. 21, n. 3, p. 97–106, set. 2018.

RAVEL, R. **Laboratório clínico: aplicações clínicas dos dados laboratoriais**. 6º ed. Rio de Janeiro: [s.n.].

REISCHL, E. et al. Distribution, adaptation and physiological meaning of thiols from vertebrate hemoglobins. **Comparative biochemistry and physiology. Toxicology & pharmacology: CBP**, v. 146, n. 1–2, p. 22–53, ago. 2007.

RENZONI, A.; ZINO, F.; FRANCHI, E. Mercury levels along the food chain and risk for exposed populations. **Environmental Research**, v. 77, n. 2, p. 68–72, maio 1998.

ROCHA, J. B. et al. Effect of treatment with mercury chloride and lead acetate during the second stage of rapid postnatal brain growth on delta-aminolevulinic acid dehydratase (ALA-D) activity in brain, liver, kidney and blood of suckling rats. **Toxicology**, v. 100, n. 1–3, p. 27–37, 26 jun. 1995.

ROELS, H. et al. Impact of air pollution by lead on the heme biosynthetic pathway in school-age children. **Archives of Environmental Health**, v. 31, n. 6, p. 310–316, dez. 1976.

ROONEY, J. P. K. The role of thiols, dithiols, nutritional factors and interacting ligands in the toxicology of mercury. **Toxicology**, v. 234, n. 3, p. 145–156, 20 maio 2007.

SANTOS, F. W. et al. Cadmium induced testicular damage and its response to administration of succimer and diphenyl diselenide in mice. **Toxicology Letters**, v. 152, n. 3, p. 255–263, 25 set. 2004.

SARAIVA, R. A. et al. Molecular docking studies of disubstituted diaryl diselenides as mammalian δ-aminolevulinic acid dehydratase enzyme inhibitors. **Journal of Toxicology and Environmental Health. Part A**, v. 75, n. 16–17, p. 1012–1022, 2012.

SASAKURA, C.; SUZUKI, K. T. Biological interaction between transition metals (Ag, Cd and Hg), selenide/sulfide and selenoprotein P. **Journal of Inorganic Biochemistry**, v. 71, n. 3–4, p. 159–162, set. 1998.

SASSA, S. Delta-aminolevulinic acid dehydratase assay. **Enzyme**, v. 28, n. 2–3, p. 133–145, 1982.

SAVEGNAGO, L. et al. Antisecretory and antiulcer effects of diphenyl diselenide. **Environmental Toxicology and Pharmacology**, v. 21, n. 1, p. 86–92, jan. 2006.

SAVEGNAGO, L. et al. Diphenyl diselenide exerts antidepressant-like and anxiolytic-like effects in mice: involvement of L-arginine-nitric oxide-soluble guanylate cyclase pathway in its antidepressant-like action. **Pharmacology, Biochemistry, and Behavior**, v. 88, n. 4, p. 418–426, fev. 2008.

SROGI, K. Developments in the determination of trace elements by atomic spectroscopic techniques. v. 41, p. 677–724, 2008.

STADTMAN, T. C. Selenium-dependent enzymes. **Annual Review of Biochemistry**, v. 49, p. 93–110, 1980.

STREETS, D. G. et al. Total Mercury Released to the Environment by Human Activities. **Environmental Science & Technology**, v. 51, n. 11, p. 5969–5977, 6 jun. 2017.

THOMPSON, J.; JONES, D. D.; BEASLEY, W. H. The effect of metal ions on the activity of delta-aminolevulinic acid dehydratase. **British Journal of Industrial Medicine**, v. 34, n. 1, p. 32–36, fev. 1977.

VAN VLEET, T. R.; SCHNELLMANN, R. G. Toxic nephropathy: environmental chemicals. **Seminars in Nephrology**, v. 23, n. 5, p. 500–508, set. 2003.

VEDAVATHI, M.; GIRISH, K. S.; KUMAR, M. K. Isolation and characterization of cytosolic alanine aminotransferase isoforms from starved rat liver. **Molecular and Cellular Biochemistry**, v. 267, n. 1–2, p. 13–23, dez. 2004.

VIRGA, R. H. P.; GERALDO, L. P.; SANTOS, F. H. Avaliação de contaminação por metais pesados em amostras de siris azuis. v. 4, n. 27, p. 779–785, 2007.

WHITEHEAD, M. W. et al. A prospective study of the causes of notably raised aspartate aminotransferase of liver origin. **Gut**, v. 45, n. 1, p. 129–133, jul. 1999.

WHO. **Inorganic mercury, Envionmental Health Criteria 118**. [s.l: s.n.].

WHO, W. H. O. Inorganic mercury. **Environmental Health Criteria**, v. 118, p. 168, 1991.

WOODLING, J. D.; BRINKMAN, S. F.; HORN, B. J. Nonuniform accumulation of cadmium and copper in kidneys of wild brown trout (Salmo trutta) populations. **Archives of Environmental Contamination and Toxicology**, v. 40, n. 3, p. 381–385, abr. 2001.

ZALUPS, R. K. Early aspects of the intrarenal distribution of mercury after the intravenous administration of mercuric chloride. **Toxicology**, v. 79, n. 3, p. 215–228, 24 maio 1993.

ZALUPS, R. K. Molecular interactions with mercury in the kidney. **Pharmacological Reviews**, v. 52, n. 1, p. 113–143, mar. 2000.

ZALUPS, R. K.; LASH, L. H. Advances in understanding the renal transport and toxicity of mercury. **Journal of Toxicology and Environmental Health**, v. 42, n. 1, p. 1–44, maio 1994.