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Suélen Osório Heck

EFEITO HIPOLIPIDÊMICO E HEPATOPROTETOR DO 4,4'-DICLORO-DIFENIL DISSELENETO EM UM MODELO DE HIPERLIPIDEMIA EM RATOS

Santa Maria, RS
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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito para obtenção do grau de **Mestre em Bioquímica Toxicológica**

Orientador: Prof. Dr. Gilson Zeni
Orientadora: Prof.ª Dr.ª Cristina Wayne Nogueira

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“Na vida, não vale tanto o que temos,
nem tanto importa o que somos.
Vale o que realizamos com aquilo que possuímos e,
acima de tudo, importa o que fazemos de nós!”

Chico Xavier

RESUMO

EFEITO HIPOLIPIDÊMICO E HEPATOPROTETOR DO 4,4'-DICLORO-DIFENIL DISSELENETO EM UM MODELO DE HIPERLIPIDEMIA EM RATOS

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ORIENTADOR: Gilson Zeni

A hiperlipidemia pode ser manifestada pela elevação de colesterol total (CT), triglicerídeos (TGs) e lipoproteínas de baixa densidade (LDL), bem como pela diminuição de lipoproteínas de alta densidade (HDL) no soro. A hiperlipidemia está associada com o estresse oxidativo e dano endotelial, seguido de ateromas e desenvolvimento de doenças cardiovasculares (DCs). Além disso, elevados níveis lipídicos estão relacionados com o desenvolvimento de Doença Hepática Gordurosa Não Alcoólica (DHGNA). Devido às limitações e efeitos colaterais dos fármacos existentes atualmente para o tratamento de dislipidemias, torna-se interessante a busca por novas drogas com potencial hipolipidêmico e hepatoprotetor. O objetivo deste trabalho foi avaliar os possíveis efeitos protetores do 4,4'-dicloro-difenil disseleneto [(*p*-ClPhSe)$_2$] sobre a ação hiperlipidêmica e hepatotóxica induzida por Triton WR – 1339 em ratos. Para esta proposta, ratos Wistar foram tratados diariamente, durante sete dias, pela via intragástrica com (*p*-ClPhSe)$_2$ na dose de 10 mg/kg ou óleo mineral (veículo). No 7º dia, trinta minutos após a última administração, os animais receberam salina (1 ml/kg, intraperitoneal, i.p.) ou triton WR-1339 (400 mg/kg, 1 ml/kg, i.p.). Após jejum de 18h, os animais foram expostos ao monitor de atividades para avaliação da atividade exploratória e locomotora. Subsequentemente, o sangue foi coletado para realização de dosagens bioquímicas. Os níveis de CT, TG, colesterol não-HDL, índice de risco coronariano, espécies reativas de oxigênio (EROS), alanina aminotransferase (ALT) e aspartato aminotransferase (AST) foram significativamente aumentados em ratos administrados com triton WR-1339. O tratamento com (p-ClPhSe)$_2$ resultou em significativo decréscimo dos parâmetros lipídicos e enzimáticos alterados por triton WR-1330. O (*p*-ClPhSe)$_2$ não foi eficaz em proteger do aumento de EROS, porém os animais tratados com o composto orgânico de selênio apresentaram aumento nos níveis de tióis não proteicos (NPSH). O (p-ClPhSe)$_2$ apresentou efeito promissor em atenuar a hiperlipidemia e danos hepáticos induzidos por triton WR-1339.

Palavras-chave: compostos orgânicos de selênio, dislipidemia, hepatotoxicidade, Triton WR – 1339, ratos.

ABSTRACT

HYPOLIPIDEMIC AND HEPATOPROTECTIVE EFFECT OF 4,4'-DICHLORO-DIPHENYL DISELENIDE IN A HIPERLIPIDEMIC MODEL IN RATS

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Hyperlipidemia can be manifested by elevation in total cholesterol (TC), triglycerides (TGs) and low density lipoprotein (LDL), as well as by reduction in high density lipoprotein (HDL) levels. Hyperlipidemia is associated with oxidative stress and endothelial damage followed by atheroma and cardiovascular disease (CVDs) development. Moreover, high lipid levels are related with non alcoholic fatty liver disease (NAFLD). In view of the limitations and side effects of current drugs to treat hyperlipidemia, it becomes interesting to search for new drugs with hypolipidemic and hepatoprotective effects. The purpose of this study was to investigate the possible hepatoprotective and antihyperlipidemic effects of 4,4'-dichlorodiphenyl diselenide [(*p*-ClPhSe)$_2$] in a hyperlipidemia model induced by Triton WR-1339 in rats. Biochemical analyses and hepatic oxidative stress parameters were evaluated in rats exposed to triton WR-1339 (400 mg/kg; i.p.) and treated with (p-ClPhSe)$_2$ (10 mg/kg; i.g.) for seven days. After triton WR-1339 injection and fasting for 18 hours, the animals were exposed to the activity monitor for evaluation of exploratory and locomotor activity. Subsequently, blood was collected for determining biochemical parameters. The levels of TC, TGs, non-HDL-cholesterol (non-HDL), coronary risk index (CRI), reactive oxygen species, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly increased in triton treated rats. (p-ClPhSe)$_2$ treatment resulted in a significant decrease in plasma lipid levels and was effective in normalizing the enzyme activities. (p-ClPhSe)$_2$ did not protect against the increase of ROS levels, but increased NPHS levels in triton treated animals. (p-ClPhSe)$_2$ could have potential in hyperlipidemia treatment as well as to reduce liver damage caused by this disorder.

Keywords: organoselenium compound; dyslipidemia; hepatotoxicity; Triton WR-1339; rats.

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LISTA DE ABREVIATURAS

$(p\text{-}ClPhSe)_2$ – 4,4'-dicloro difenil disseleneto
$(PhSe)_2$ – Difenil disseleneto
ACAT- Acil-CoA-colesterol aciltransferase
AG- Ácidos graxos
Apo- Apolipoproteínas
CT- Colesterol total
DAC- Doenças arteriais coronarianas
DC- Doenças cardiovasculares
DHGNA- Doença hepática gordurosa não alcoólica
EC- Ésteres de colesterol
EHNA- Esteato-Hepatite Não Alcoólica
EROS- Espécies reativas de oxigênio
HDL- Lipoproteínas de alta densidade
HMG-CoA- 3-hidróxi-3-metilglutaril coenzima A
IDL- Lipoproteínas de densidade intermediária
LCAT- Lecitina-colesterol aciltransferase
LDL- Lipoproteína de baixa densidade
LDN- Lipogênese *de novo*
LPL- Lipase lipoproteica
QM - Quilomicrons
RLDL- Receptor da LDL
TG- Triglicerídeos
VLDL- Lipoproteínas de muito baixa densidade

SUMÁRIO

1 INTRODUÇÃO

1.1 Lipídeos

Os lipídeos são moléculas hidrofóbicas e de estrutura diversificada com inúmeras funções biológicas, tais como fornecimento de energia, mediação da sinalização celular e auxílio no processo de formação da membrana plasmática e outras organelas celulares. Clinicamente, os lipídeos de maior relevância são o colesterol e os triglicerídeos (TG) (BROWN e GOLDSTEIN, 1986; DUNNING et al., 2014; OH et al., 2006).

O colesterol total (CT) plasmático pode ser dividido em colesterol livre e ésteres de colesterol (EC), que representa 70% do colesterol total (BROWN et al., 1981). O colesterol, além de exercer papel importante na manutenção da integridade celular e fluidez das membranas biológicas, é um precursor essencial para a síntese de moléculas bioativas tais como ácidos biliares, óxiesterois e hormônios esteróides, as quais auxiliam na regulação do metabolismo e na comunicação intra e extracelular (BROWN e GOLDSTEIN, 1986; HEGELE, 2009; LIU, 2009) (Figura 1). O colesterol também é necessário para o desenvolvimento embrionário e fetal (WOOLLETT, 2011). Devido à grande importância, é preciso manter níveis de colesterol suficientes a fim de satisfazer estas necessidades, porém esses níveis devem ser mantidos estreitos, já que o excesso de colesterol livre é tóxico para as células (TABAS, 2002).

Os níveis de lipídeos plasmáticos são determinados pela absorção exógena e pela síntese endógena (XIE et al., 2007). Dessa maneira, o colesterol pode ser sintetizado no fígado a partir da molécula de acetil CoA (síntese endógena) (Figura 2) ou derivado de fontes dietéticas (REPA e MANGELSDORF, 2000), sendo estimada uma proporção de 70:30 a contribuição da síntese endógena contra a contribuição por ingestão respectivamente (GRUNDY, 1983). O fígado é um órgão importante para a manutenção dos níveis de colesterol e para a síntese de diversas proteínas e enzimas relacionadas a homeostase lipídica, como lecitina-colesterol aciltransferase (LCAT), receptor da LDL (RLDL), 3-hidróxi-3-metilglutaril coenzima A (HMG-CoA) redutase e acil- CoA-colesterol aciltransferase (ACAT) (BATE et al., 2007). A LCAT catalisa a esterificação do colesterol livre nas lipoproteínas

plasmáticas e desempenha um papel crítico no metabolismo de HDL, auxiliando na remoção do excesso de colesterol dos tecidos periféricos com subsequente condução ao fígado para posterior excreção biliar (KUNNEN e VAN ECK, 2012). Outra enzima de bastante importância é a Lipase Lipoproteica (LPL, "*lipoprotein lípase"),* que é responsável pela hidrólise dos TG, produzindo quilomícrons (QM) remanescentes e lipoproteínas de densidade intermediária (IDL, "intermediate-density lipoproteins"), que normalmente são depurados rapidamente da corrente sanguínea para o fígado (RENSEN et al., 1997).

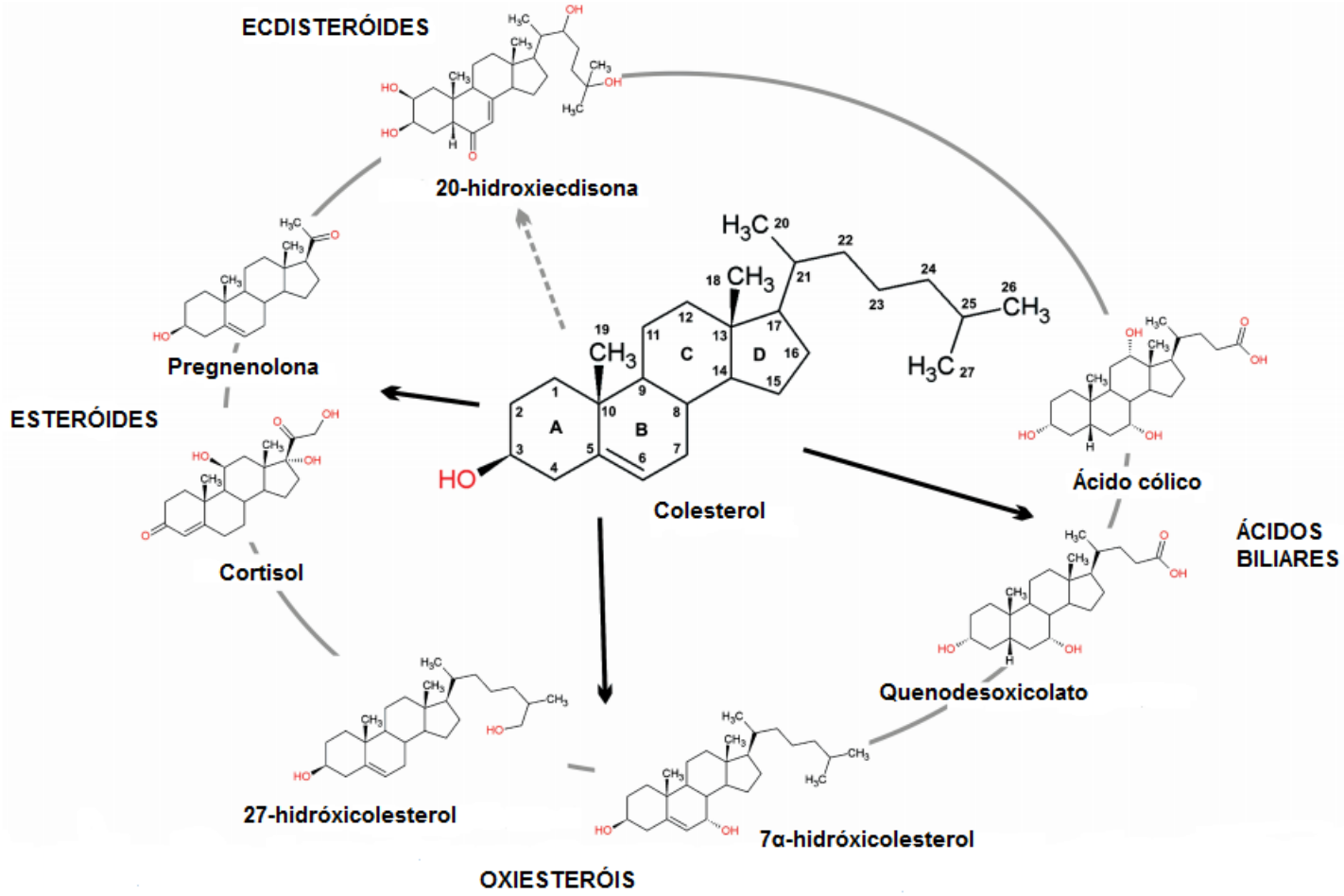

Figura 1. Colesterol e seus metabólitos. Adaptado de (MIDZAK e PAPADOPOULOS, 2014).

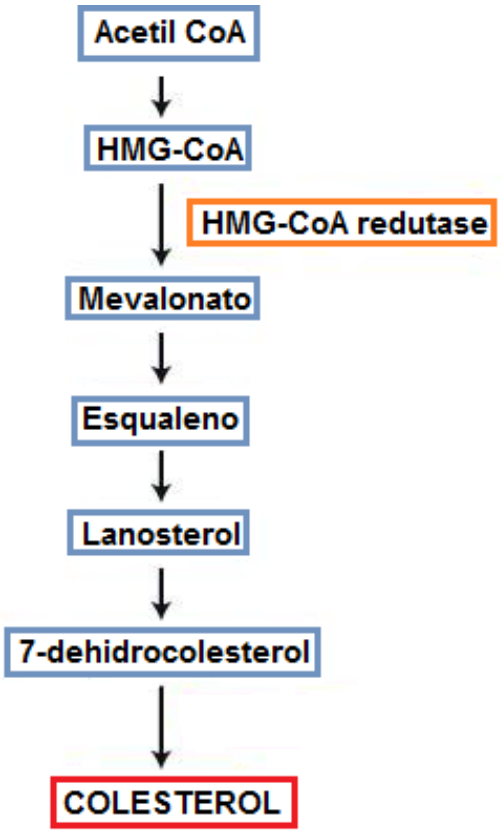

Figura 2. Biossíntese do colesterol. Adaptado de (VANCE, 2012).

Os TG são moléculas constituídas pela ligação éster de três ácidos graxos (AG) a uma molécula de glicerol (HEGELE, 2009), que podem ser obtidos a partir da dieta ou da síntese endógena, sendo que os TG constituem 90% dos lipídeos da dieta (AIZAWA et al., 2015). A síntese endógena de TG é um processo importante e estritamente regulado que ocorre principalmente no tecido adiposo, mas também no fígado, músculo, coração e pâncreas. Essa via de síntese é utilizada com o objetivo de manter e controlar a homeostase de energia. TG são sintetizados a partir de AG derivados da dieta, da lipólise periférica ou ainda da lipogênese *de novo* (LDN). A lipólise ocorre principalmente no tecido adiposo e é caracterizada pela hidrólise de TG, que resulta na liberação de AG e glicerol na circulação (SAPONARO et al., 2015). A LDN ocorre principalmente no fígado após uma refeição rica em carboidratos, onde apenas uma fração de carboidratos é armazenada como glicogênio hepático e a restante é convertida em AG e TG (DONNELLY et al., 2005; HELLERSTEIN, 1999).

Devido à baixa solubilidade dos lipídeos no sangue, faz-se necessário a presença de lipoproteínas para transporta-los pela corrente sanguínea. As lipoproteínas possuem um núcleo hidrofóbico rodeado por uma monocamada de fosfolípidios polares na superfície (BROWN et al., 1981) (figura 3), e são geralmente classificadas com base na densidade flutuante, sendo divididas em cinco classes: quilomicrons (QM), lipoproteínas de baixa densidade (LDL, "*low-density lipoproteins*"), lipoproteínas de muito baixa densidade (VLDL, "*very low-density lipoproteins*"), lipoproteínas de densidade intermediária (IDL, "*intermediate-density lipoproteins*") e lipoproteínas de alta densidade (HDL, "*high-density lipoproteins*). As principais características dessas lipoproteínas estão representadas na tabela 1, onde é possível observar que as QM e as VLDL são ricas em TG, sendo consideradas portanto as principais carregadoras dos TG, enquanto que as LDL e as HDL contém colesterol abundante em suas estruturas, sendo essas as principais carregadoras de colesterol. As HDL são responsáveis pelo transporte reverso do colesterol, ou seja promovem o efluxo do colesterol em excesso das paredes arteriais para o fígado, que posteriormente será excretado pela bílis (REICHL e MILLER, 1989). O transporte de lipídeos realizado pelas lipoproteínas está representado na figura 4.

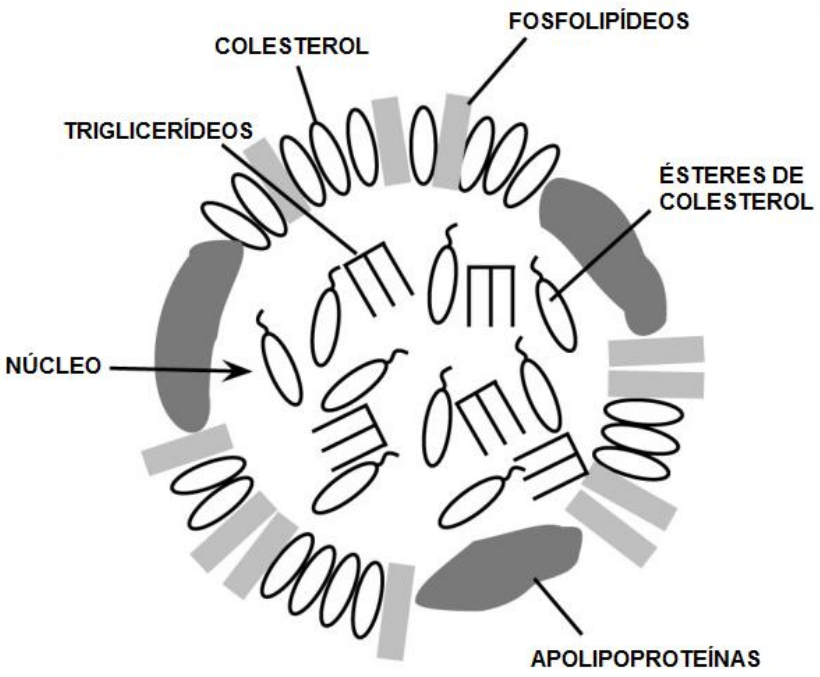

Figura 3. Estrutura geral das lipoproteínas. Adaptado de (WADE et al., 2014).

Tabela 1. Classificação e características das lipoproteínas

Classe	Densidade	Proteínas	Colesterol total	Fosfolipídeos	Triglicerídeos	Ácidos graxos
	mg/dL	%	%	%	%	%
QM	< 0,95	< 2	4-8	7-8	84-88	0
VLDL	0,950-1,006	7-10	20-25	18-20	50-55	1
IDL	1,006-1,019	10-18	29-45	22-27	25-31	1
LDL	1,019-1,063	20-25	45-58	20-28	10-15	1
HDL	1,063-1,210	33-57	17-40	26-46	3-15	0-6

Adaptado de (AIZAWA et al., 2015)

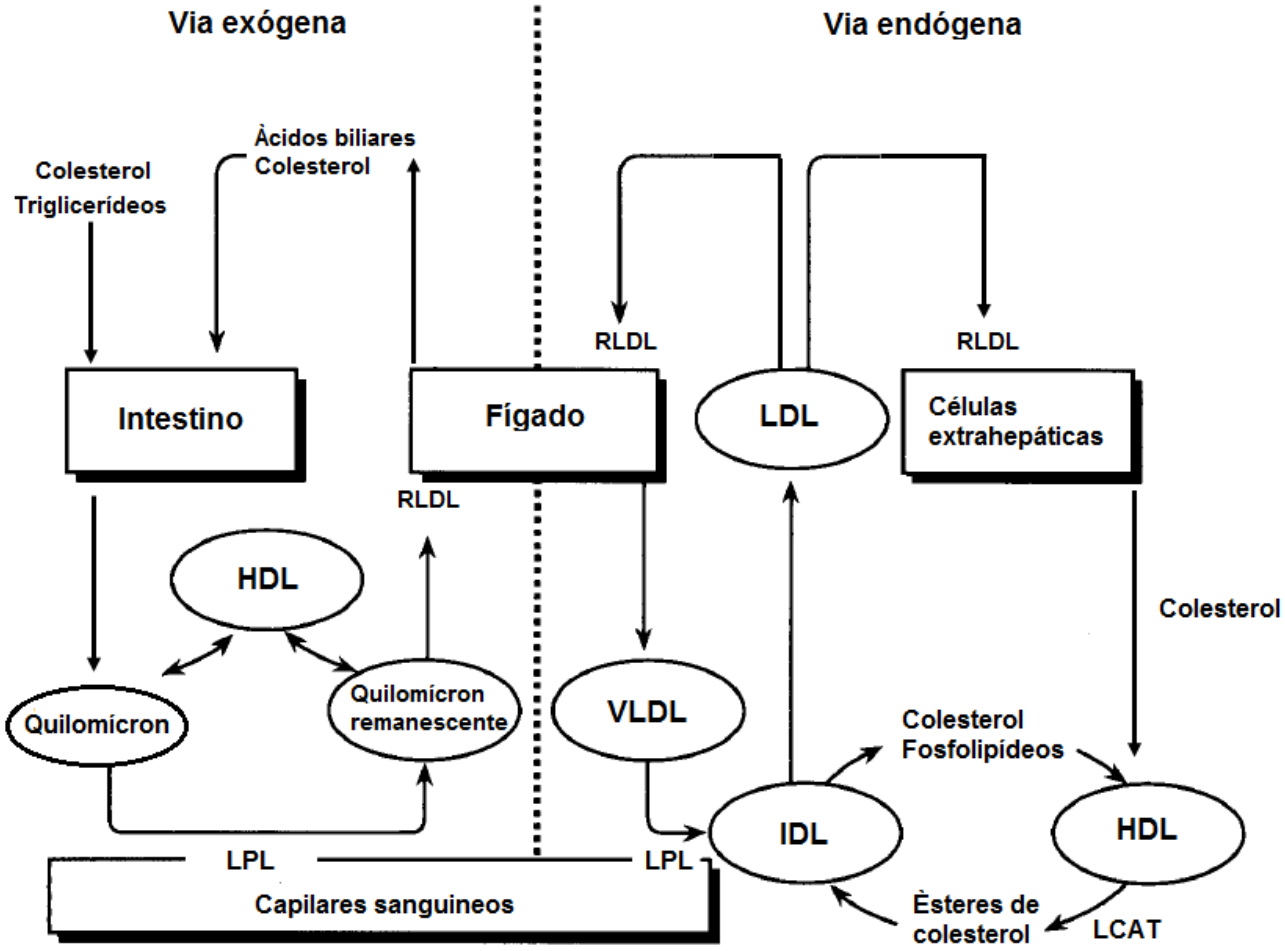

Figura 4. Transporte de lipídios pelas lipoproteínas. Adaptado de (RUSSELL, 1992).

Na superfície das lipoproteínas existem as apolipoproteínas (Apo). Essas são proteínas anfipáticas que ajudam a estabilizar a estrutura das lipoproteínas e desempenham um papel fundamental no metabolismo das mesmas, servindo como

receptores de ligantes, cofatores de enzimas e transportadoras de lipídeos. As Apo são classificadas em apo A, apo B, apo C, ou apo E. Dentro dessa classificação, ainda podem ser divididas com base em características biológicas e estruturais, nomeadamente como permutáveis e não permutáveis (AIZAWA et al., 2015). As propriedades e funções das principais Apo estão representadas na tabela 2.

Tabela 2. Principais apolipoproteínas e suas funções

Nome	Lipoproteína associada	Principal função
Apo A-I	HDL >> QM	Cofator da enzima LCAT
Apo A-II	HDL	Inibição da enzima LCAT
Apo B-48	QM e QM remanescente	Formação de QM
Apo B-100	VLDL, IDL e LDL	Formação de VLDL/LDL
		Ligante do RLDL
Apo C-I	QM, VLDL, IDL e HDL	Inibição da proteína transportadora de ésteres de colesterol
Apo C-II	QM, VLDL, IDL e HDL	Cofator da LPL
Apo C-III	QM, VLDL, IDL e HDL	Inibição da LPL
		Formação e secreção de VLDL
Apo E	QM, QM remanescente, VLDL, IDL e HDL	Ligante do RLDL

Adaptado de (AIZAWA et al., 2015)

1.2 Hiperlipidemia

As dislipidemias são distúrbios que ocorrem devido às alterações do metabolismo de lipoproteínas, sejam essas alterações relacionadas com deficiência ou superprodução. A hiperlipidemia pode ser manifestada pela elevação de CT, TG e LDL, sendo essas alterações isoladas ou associadas, bem como pela diminuição de HDL no soro (AHMED et al., 1998; GOTTO, 2005).

O aumento de lipídeos plasmáticos pode ser causado por disfunção genética, por drogas, por doenças ou pela dieta (RAASCH, 1988). Diante disso, a hiperlipidemia é classificada em primária e secundária. A primeira desenvolve-se a partir de alterações genéticas, como exemplo tem-se a hipercolesterolemia e a hipertrigliceridemia familiar. A segunda dá-se quando a hiperlipidemia ocorre como uma consequência de doenças (ex.: diabetes mellitus, hipotireoidismo, falência

hepática e renal) de tratamentos (ex.: glicocorticóides, retinóides e betabloqueadores) ou de hábitos de vida inadequados (ex.: dieta, tabagismo e etilismo) (IUGHETTI et al., 2010; O'GORMAN et al., 2011).

A hiperlipidemia está associada com o estresse oxidativo e doenças cardiovasculares (DC). O estresse oxidativo é definido como um desequilíbrio entre agentes oxidantes e antioxidantes, ocorrendo predomínio de oxidantes, e é considerado o principal fator relacionado à etiologia e desenvolvimento de alterações na parede arterial. As espécies reativas de oxigênio (EROS) são moléculas que funcionam como sinalizadores vasculares e mediadoras de estresse oxidativo. O aumento de EROS está relacionado com níveis de lipídeos plasmáticos elevados (CASTELLON e BOGDANOVA, 2013; OHARA et al., 1993). Distúrbios do metabolismo lipídico seguido de estresse oxidativo são os principais fatores de risco para o surgimento e progressão de DC (ALI et al., 2010; O'GORMAN et al., 2011).

Níveis lipídicos plasmáticos elevados podem resultar em dano endotelial, que acomete a camada íntima dos vasos sanguíneos, dificultando a circulação de sangue. Esse dano ocorre principalmente devido á agregação e depósito de lipoproteínas nas paredes dos vasos sanguíneos, seguido de uma cascata de respostas, incluindo a oxidação das lipoproteínas e quimiotaxia celular, que levará à lesão (GRAHAM et al., 2012; STEYERS e MILLER, 2014). A presença de disfunção endotelial independentemente da sua etiologia é um evento central que dá origem ao processo aterosclerótico (IUGHETTI et al., 2010; SATTAR, 2004). A aterosclerose é uma doença inflamatória progressiva caracterizada pelo acúmulo de lipídeos e elementos fibrosos na parede arterial, que pode resultar em doenças arteriais periféricas, doenças cerebrovasculares e doenças arteriais coronarianas (DAC) (LUSIS, 2000; ROSS, 1993). As consequências da aterosclerose, responsáveis pelo desenvolvimento de DC, estão entre as principais causas de morbidade e mortalidade mundial. DAC é um termo médico que se refere a um conjunto de condições responsáveis pelo estreitamento e endurecimento dos vasos sanguíneos, que geralmente tem como principal consequência morte súbita (CASTELLON e BOGDANOVA, 2013; LANGLOIS, 2012; RINKUNIENE et al., 2009). O desenvolvimento das DAC está relacionado e aumenta significantemente de acordo com a idade, histórico familiar, obesidade, diabetes, aumento da pressão arterial e/ou hipercolesterolemia (BARRETT-CONNOR e KHAW, 1984; HUBERT et al., 1983; JOUSILAHTI et al., 1999; KANNEL e MCGEE, 1979; WILSON et al.,

1998). Além disso, níveis elevados de TG plasmáticos frequentemente estão associados com fatores de risco para o desenvolvimento das DAC (YUAN et al., 2007). Estima-se que o aumento de 1mmol/L de TG no plasma pode evoluir para 76% o risco de desenvolver DC em mulheres e 31% em homens (AUSTIN, 1999).

Além de estar relacionada com o desenvolvimento de doenças cardiovasculares, a hiperlipidemia também pode causar alterações no fígado. Níveis elevados de colesterol podem levar ao acúmulo de gotículas de lipídeos no fígado, resultando em dano hepático, como a esteatose (JEONG et al., 2005). Além disso, a hiperlipidemia está relacionada com redução do sistema de defesa antioxidante (KUMAR et al., 2006; OH et al., 2006) e desenvolvimento de Doença Hepática Gordurosa Não Alcoólica (DHGNA). A DHGNA afeta de 10 a 35% da população mundial atual, e é caracterizada pelo excesso de gordura depositada no fígado (principalmente na forma de TG, assim a DHGNA abrange um amplo espectro de problemas hepáticos como a esteatose hepática, fibrose, cirrose e Esteato-Hepatite Não Alcoólica (EHNA) (GAGGINI et al., 2013; YKI-JARVINEN, 2014). Esse acúmulo de gordura hepática é resultante do desequilíbrio entre o fornecimento lipídico (exógeno ou endógeno – LDN) e a remoção lipídica (oxidação de AG ou secreção de lipoproteínas ricas em TG - VLDL) (Figura 5) (FERRAMOSCA e ZARA, 2014).

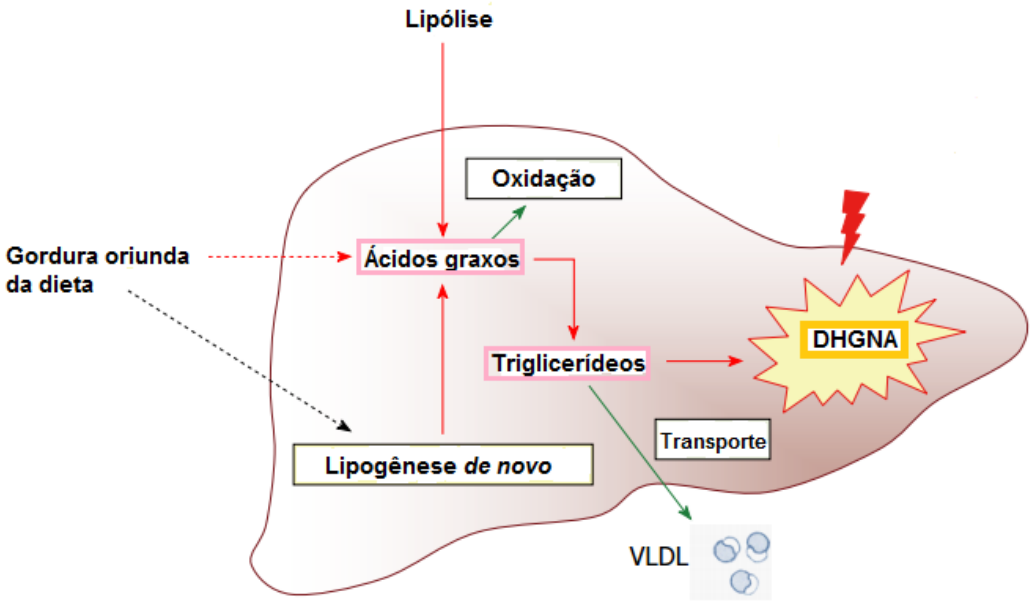

Figura 5. Vias metabólicas contribuintes para o desenvolvimento de DHGNA. Adaptado de (FERRAMOSCA e ZARA, 2014).

1.3 Estatinas

As estatinas estão entre os medicamentos mais utilizados e eficazes para reduzir elevados níveis de colesterol plasmático. Mundialmente, mais de 200 milhões de pessoas fazem uso dessa classe de fármaco, sendo mais de 30 milhões de usuários nos EUA (BLAHA e MARTIN, 2013).

Entre as principais representantes das estatinas, encontram-se a sinvastatina, a atorvastatina, a pravastatina, a fluvastatina, a rosuvastatina, e a lovastatina, que agem através da inibição da enzima HMG-CoA (hidroximetilglutaril coenzima A) redutase, que é essencial para a produção do colesterol. Essa enzima catalisa a conversão da HMG-CoA em mevalonato, substrato para a síntese do colesterol (Figura 6). A inibição da HMG-CoA redutase tem como resultado a diminuição da síntese hepática do colesterol e o aumento da síntese de receptores LDL na superfície do hepatócito. Consequentemente, ocorre aumento da remoção de LDL do plasma, declínio dos níveis plasmáticos de colesterol e decréscimo de sua absorção intestinal (BROWN e GOLDSTEIN, 1980; VEILLARD e MACH, 2002). Além de reduzir os níveis de LDL, as estatinas também possuem efeitos diretos sobre a placa aterosclerótica, levando à estabilização coronariana e até mesmo à modesta regressão do ateroma (NICHOLLS et al., 2011).

Apesar dos efeitos benéficos, o uso das estatinas está associado à ocorrência de efeitos colaterais indesejáveis. Entre os mais comuns, tem-se a miopatia. A rabdomiólise é a manifestação mais grave de miopatia associada às estatinas (DESAI et al., 2014). Além disso, estudos relatam um aumento consistente no risco de diabetes associado à terapia com estatina. Dados experimentais sugerem que as estatinas podem reduzir a função e promover apoptose de células β pancreáticas, levando à diminuição de secreção da insulina (KOH et al., 2009; NAVARESE et al., 2014). Estudos também apresentam associação entre o uso de estatinas e a diminuição da função cognitiva (WAGSTAFF et al., 2003).

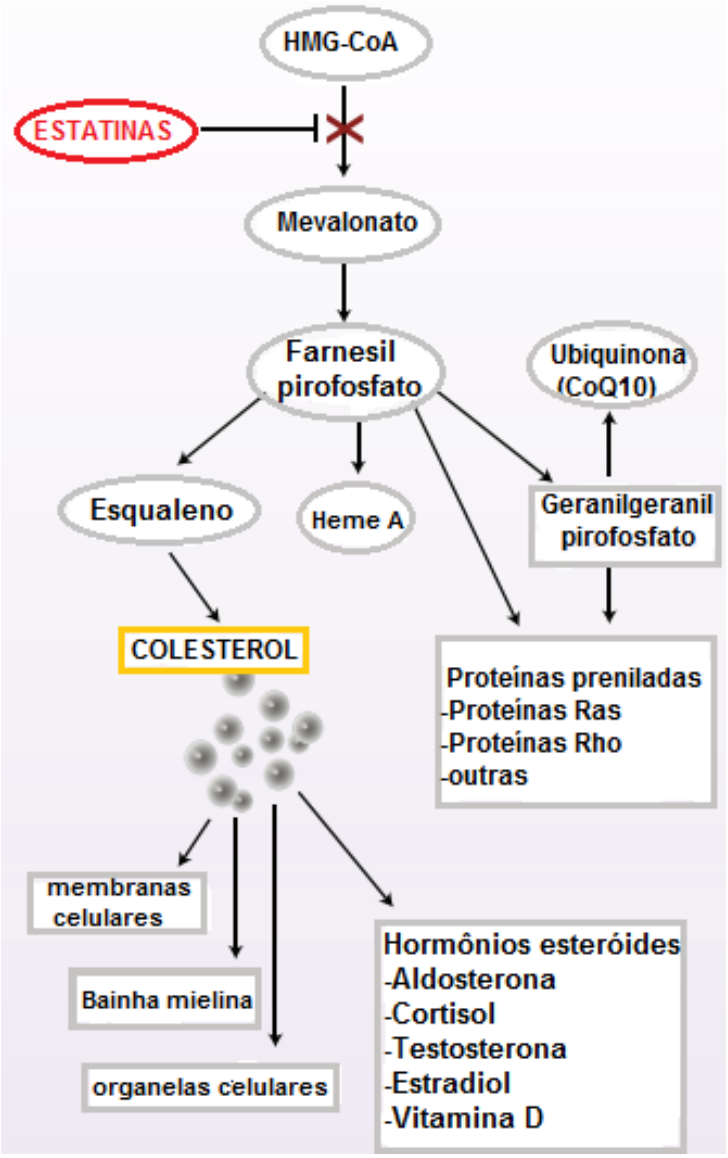

Figura 6. Ação das estatinas. Adaptado de (O'GORMAN et al., 2011).

Além dos efeitos indesejáveis, a terapia com estatinas torna-se limitada, pois não é recomendável a utilização dessa classe de fármacos durante os períodos de puberdade, gestação e lactação (BRUCKERT et al., 2005; FISCHER et al., 2015; JOY e HEGELE, 2009; THOMPSON et al., 2006; WANG et al., 2008). Além disso, embora as estatinas sejam bastante utilizadas, muitos pacientes não alcançam o objetivo de reduzir os níveis plasmáticos de LDL durante a terapia. Assim, as taxas de risco de doenças cardíacas coronárias e mortalidade prevalecem altas (WATERS et al., 2009).

1.4 Selênio e compostos orgânicos de selênio

Selênio (Se) é um elemento traço essencial do ponto de vista nutricional, estando naturalmente presente em muitos alimentos como a castanha-do-pará, alho, cebola, brócolis, cereais, ovos e carnes. O seu efeito biológico é exercido via incorporação em selenoproteínas, que desempenham um papel importante sobre metabolismo dos hormônios tireoidianos, síntese de DNA, proteção contra estresse oxidativo e inflamação (DUMONT et al., 2006; TOUAT-HAMICI et al., 2014). Há evidências que relacionam a deficiência de Se com o desenvolvimento de muitas doenças crônicas, incluindo doenças cardiovasculares (ALEHAGEN e AASETH, 2015; SABINO et al., 2013), câncer (FACOMPRE e EL-BAYOUMY, 2009; SUN et al., 2013) e diabetes (MAO et al., 2014; RAYMAN e STRANGES, 2013; ROCOURT e CHENG, 2013). Em detrimento disso, a Organização Mundial de Saúde (OMS) recomenda uma ingestão diária de 34-35 µg para adultos (FAO/OMS, 2002), seja através da ingestão de alimentos comuns, de origem animal e vegetal, ou através de suplementação (DUMONT et al., 2006; RAYMAN, 2008). Além disso, a biodisponibilidade do Se varia de acordo com a fonte e estado nutricional do indivíduo, sendo significativamente maior para as formas orgânicas de selênio (KIM e MAHAN, 2001; YOUNG et al., 1982). Em função disso, compostos orgânicos de Se têm sido amplamente estudados pelo nosso e por outros grupos de pesquisa, uma vez que estas moléculas apresentam uma diversidade de aplicações biológicas (NOGUEIRA e ROCHA, 2011; NOGUEIRA, C. W. et al., 2004).

Evidências sugerem a existência de efeitos anti-hiperlipidêmicos de compostos orgânicos de Se. Estudos mostraram proteção do composto ebselen contra a oxidação lipídica LDL induzida por cobre e radical peroxil (LASS et al., 1996), e esse mesmo composto apresentou redução de lesões ateroscleróticas em camundongos diabéticos apoE-/- (CHEW et al., 2009). O composto disseleneto de difenila [$(PhSe)_2$] apresentou efeitos benéficos contra a oxidação induzida por íons cobre ou gerador de radicais hidroxil no soro humano, na LDL isolada e em fatias de aorta animal (DE BEM et al., 2008). Além disso, o $(PhSe)_2$ apresentou atividade do tipo hipocolesterolêmica em coelhos (DE BEM et al., 2009) e em camundongos (DA ROCHA et al., 2009). Esse mesmo composto também apresentou atividade em reduzir lesão aterosclerótica de camundongos hipercolesterolemicos LDLr-/-, através da diminuição do processo inflamatório e do estresse oxidativo (HORT et al., 2011).

A introdução de dois átomos de cloro nos anéis aromáticos do $(PhSe)_2$ resulta em um composto orgânico de selênio chamado 4,4'-dicloro-difenil disseleneto [(*p*-$ClPhSe)_2$] (Figura 7). Estudos demonstraram que o (*p*-$ClPhSe)_2$ apresentou menor toxicidade hepática e renal em camundongos expostos ao cloreto de mercúrio quando comparado ao $(PhSe)_2$ (DE FREITAS et al., 2012) Além disso, estudos realizados em nosso laboratório demonstram que a DL 50 de uma administração aguda de $(PhSe)_2$ em camundongos é >312 mg/kg e de (*p*-$ClPhSe)_2$ é >381 mg/kg (NOGUEIRA e ROCHA, 2010), indicando que o (*p*-$ClPhSe)_2$ apresenta menor toxicidade quando comparado ao $(PhSe)_2$.

O (*p*-$ClPhSe)_2$ apresentou atividade protetora *in vitro* e *in vivo* contra dano oxidativo cerebral induzido por nitroprussiato de sódio (PRIGOL et al., 2009). Estudos demonstraram que, além de apresentar atividade antioxidante e antidepressiva em ratos velhos, o (*p*-$ClPhSe)_2$ teve efeito em melhorar o prejuízo cognitivo causado pelo envelhecimento nesses animais (BORTOLATTO et al., 2012). Além disso, foi relatada atividade do tipo anorexígena do (*p*-$ClPhSe)_2$ mediada pelo sistema serotoninérgico com saciedade comportamental precoce dissociada dos principais efeitos tóxicos (BORTOLATTO et al., 2015) e efeito homeostático no metabolismo da glicose alterado pela administração de glutamato monossódico em ratos (QUINES et al., 2016).

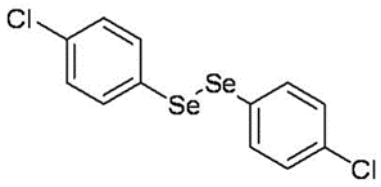

Figura 7. Estrutura química do composto (p-$ClPhSe)_2$.

1.5 Triton WR-1339

O triton WR-1339, um detergente não iônico, tem sido utilizado em modelos animais para induzir hiperlipidemia aguda e aterosclerose (HARNAFI et al., 2008). O mecanismo de ação desse agente hiperlipidêmico está relacionado principalmente à inibição da atividade da enzima lipase lipoproteica (LPL) (HAYASHI et al., 1981). A LPL desempenha um papel importante na regulação do metabolismo lipídico. É

sintetizada em células parenquimais de muitos tecidos, porém exerce suas funções na superfície luminal do endotélio vascular. Sua principal função está relacionada com a hidrólise de TG séricos derivados de lipoproteínas ricas em TG (QM e VLDL). (CUNNINGHAM e ROBINSON, 1969; WION et al., 1987). Assim, a diminuição ou o bloqueio da LPL impede o retorno das lipoproteínas e/ou seus produtos de degradação ao fígado, o que acarreta em estímulo na biossíntese do colesterol (FRANTZ e HINKELMAN, 1955). Dessa forma, após a injeção de triton WR-1339 ocorre aumento de secreção de VLDL pelo fígado e redução do catabolismo de VLDL e LDL, assim os níveis plasmáticos de CT e TG tornam-se elevados (OTWAY e ROBINSON, 1967).

Além de induzir hiperlipidemia, estudos recentes mostraram níveis elevados de enzimas marcadoras de dano hepático em ratos tratados com triton WR-1339 (ANANDHI et al., 2013), o que indica hepatotoxicidade induzida por esse detergente.

Considerando a alta prevalência de hiperlipidemia e doenças relacionadas a esta, torna-se importante a busca por novas drogas com potencial hipolipidêmico.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Considerando os aspectos mencionados, o objetivo desse estudo foi investigar os possíveis efeitos protetores do $(p\text{-ClPhSe})_2$ em um modelo agudo de hiperlipidemia induzido por triton-WR 1339 em ratos.

2.2 OBJETIVOS ESPECÍFICOS

- Investigar os efeitos hipolipidêmico e hepatoprotetor do $(p\text{-ClPhSe})_2$ em um modelo de hiperlipidemia induzido por triton WR-1339 em ratos;
- Avaliar os efeitos do $(p\text{-ClPhSe})_2$ sobre os parâmetros de estresse oxidativo hepático após a injeção de triton WR-1339.

3 RESULTADOS

Os resultados que fazem parte dessa dissertação estão apresentados na forma de um manuscrito. Os itens introdução, materiais e métodos, resultados, discussão e referências bibliográficas do manuscrito estão dispostos de acordo com a recomendação do periódico científico no qual está submetido.

3.1 MANUSCRITO

Hypolipidemic and hepatoprotective effects of 4,4'-dichlorodiphenyl diselenide in a model of hyperlipidemia in rats

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ABSTRACT

Context: Hyperlipidemia contributes to the development of atherosclerosis, coronary heart diseases and liver fatty infiltration.

Objective: To analyze the hepatoprotective and antihyperlipidemic effects of $(p\text{-ClPhSe})_2$ in a hyperlipidemia model induced by Triton WR-1339.

Materials and methods: Biochemical analyses and hepatic oxidative stress parameters were evaluated in rats exposed to triton WR-1339 (400 mg/kg; i.p.) and treated with $(p\text{-ClPhSe})_2$ (10 mg/kg; i.g.) for seven days.

Results: The levels of total cholesterol, triglycerides, non-HDL-cholesterol, coronary risk index, ROS, alanine aminotransferase and aspartate aminotransferase activities were significantly increased in triton treated rats. $(p\text{-ClPhSe})_2$ treatment resulted in a significant decrease in plasma lipid levels and was effective in normalizing the enzyme activities. $(p\text{-ClPhSe})_2$ did not protect against the increase of ROS levels, but increased NPHS levels in triton treated animals.

Discussion and conclusion: Selenium is now a well-established essential micronutrient for mammalian species, required for several biological functions. Due to the importance of this micronutrient, organoselenium compounds have been extensively studied. In line with this, $(p\text{-ClPhSe})_2$ could have potential in hyperlipidemia treatment as well as to reduce liver damage caused by this disorder.

Keywords: organoselenium compounds; dyslipidemia; hepatotoxicity; Triton WR- 1339.

1. Introduction

Multiple mechanisms have been proposed to account for the elevated risk associated with increased triglyceride and cholesterol levels, including smooth muscle and endothelial damage, increased lipid accumulation in the arterial wall, impaired vascular repair, and abnormal plasma lipoprotein content and transport (Graham et al., 2012). Furthermore, hypercholesterolemia could also lead to the accumulation of lipid droplets in the liver, resulting in hepatic damage, such as steatosis (Jeong et al., 2005). Fatty liver–induced steatohepatitis, a specific pattern of injury within the spectrum of non-alcoholic fatty liver disease, can gradually lead to the development of fibrosis, cirrhosis, liver failure, and even hepatocellular carcinoma (Souza et al., 2012).

Hyperlipidemia is considered as a risk factor for fatty infiltration in the liver (Assy et al., 2000, Ludwig et al., 1980), besides it significantly contributes to the manifestation and development of atherosclerosis and coronary heart diseases, the latter being one of the most common causes of morbidity and mortality in the United States and worldwide (Yokozawa et al., 2003, Szapary and Rader, 2004).

Statins are the most widespread and effective drugs lowering cholesterol levels, claimed to have a number of synergetic effects (Wang et al., 2008). Despite the widespread use of statin therapy, alone or in combination with other agents, many individuals do not achieve low-density lipoprotein (LDL) goals, and the rates of risk of cardio-vascular events and mortality remain high (Waters et al., 2009).

Selenium is an essential micronutrient involved in the maintenance of several physiological functions especially due to its beneficial role (Stazi and Trinti, 2008). In this way, organoselenium compounds have emerged as a new therapeutic approach for many disorders (Nogueira and Rocha, 2011). Diphenyl diselenide [$(PhSe)_2$] has been reported to

reduce hypercholesterolemia in cholesterol-fed rabbits (de Bem et al., 2009) and in triton WR-1339 induced hyperlipidemic mice (da Rocha et al., 2009). Moreover, $(PhSe)_2$ has been shown to inhibit human LDL oxidation in vitro (de Bem et al., 2008), reduce foam atherosclerotic lesion in hypercholesterolemic LDL receptor knockout (LDLr -/-) mice, decrease infiltration of inflammatory cells in vessel-wall, and prevent the upregulation of the proatherogenic monocyte chemoattractant protein-1 (Hort et al., 2011). The molecular effects of $(PhSe)_2$ on cholesterol cell metabolism, investigated in HepG2 cells line, involve the increase in 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) phosphorylation/inactivation induced by AMP-activated kinase (AMPK) activation, and the rise in low-density lipoprotein receptors (LDLr) protein levels without directly inhibiting HMGR activity (da Rocha et al., 2013).

The introduction of a functional group (chloro) in the aromatic ring of $(PhSe)_2$ results in another compound called 4,4'-dichlorodiphenyl diselenide [$(p\text{-}ClPhSe)_2$] (Fig. 1). This analogue of $(PhSe)_2$ has been reported to be effective in preventing cerebral damage in vitro in mice (Prigol et al., 2009) and improving memory in old rats (Bortolatto et al., 2012). Moreover, $(p\text{-}ClPhSe)_2$ has been shown to have an anorectic-like action in rats (Bortolatto et al., 2015).

Triton WR-1339, a non-ionic detergent, has been used widely to produce acute hyperlipidemia and atherosclerosis pathogenesis in animals (Harnafi et al., 2008). Moreover, recent study showed elevated activities of levels in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) hepatic enzymes in triton WR-1339 treated rats (Anandhi et al., 2013), showing hepatotoxicity induced by this detergent. Based on that, the aim of the study was to investigate the possible hepatoprotective and antihyperlipidemic effects of $(p\text{-}ClPhSe)_2$ in the hyperlipidemic model induced by triton WR- 1339 in rats.

2. Materials and Methods

2.1. Animals

The experiments were conducted using adult male Wistar rats (180 – 220g) maintained at 22–25 °C with free access to water and food during treatment, under a 12:12 h light/dark cycle, with lights turned on at 7:00 A.M. All manipulations were carried out between 08:00 A.M. and 04:00 P.M. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil (#501/2015). All efforts were made to minimize animals suffering and reduce the number of animals used in the experiments.

2.2. Drugs

Triton WR-1339 (Tyloxapol) was purchased from Sigma (St. Louis, MO, USA) and dissolved in saline 0.9%.

$(p\text{-ClPhSe})_2$ was synthesized and characterized by the method previously described by Paulmier (Paulmier, 1986). The chemical purity of the studied compound (99.9 %) was determined by gas chromatography–mass spectrometry and nuclear magnetic resonance. This organoselenium compound was dissolved in mineral oil.

Simvastatin was of analytical grade and obtained from standard commercial suppliers. It was mixed with 1% carboxymethyl cellulose (CMC) and 5% Tween-80 resulting in a homogeneous suspension. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.3. Experimental design

The experimental design of this study was performed as shown in Fig. 2. The animals were divided in five groups (n=10) as follow:

Group I: mineral oil + saline

Group II: mineral oil + triton WR-1339

Group III: [$(p\text{-ClPhSe})_2$] (10 mg/kg) + saline

Group IV: [$(p\text{-ClPhSe})_2$] (10 mg/kg) + triton WR-1339

Group V: Simvastatin (20 mg/kg) + triton WR-1339

The animals received $(p\text{-ClPhSe})_2$ at a dose of 10 mg/kg (Bortolatto et al., 2012) or mineral oil (1 ml/kg per body weight) by the intragastric route once a day for seven days. Simvastatin, used as a positive control, was administered by the intragastric route to rats at a dose of 20 mg/kg for seven days, based on a dose-response curve carried out in our laboratory (data not shown). Thirty minutes after the last treatment, rats received saline (1 ml/kg, intraperitoneal, i.p.) or triton WR-1339 (400 mg/kg, 1 ml/kg, i.p.) injection (Schurr et al., 1972).

Animals were fasted for 18h and challenged in an activity chamber apparatus to evaluate locomotor and exploratory activities. Subsequently, blood samples were collected by heart punction in anaesthetized animals, using heparin as anticoagulant, and plasma was separated by centrifugation (1500 g) for 10 min. After that, the rats were killed by decapitation and the livers were removed and homogenized in 50 mM Tris-HCl, pH 7.4. The homogenate was centrifuged at 1500 g at 4°C for 10 min and a low-speed supernatant fraction (S1) was used for assays.

2.4. Body weight gain

The body weight of rats (g) was recorded every day during seven days of treatment. The individual body weight gain was recorded and calculated according to the formula

[baseline body weight (obtained before the beginning of treatment) — body weight at the end of the experiment].

2.5. Biochemical analysis in plasma

The plasma levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using commercially available assay kits (Labtest Diagnostica, MG, Brazil). Non-HDL-cholesterol (Non-HDL) values were obtained by the difference between total cholesterol and HDL-cholesterol levels (da Rocha et al., 2009). The coronary risk index (CRI) was calculated as: TC/HDL (Alladi and Radha Shanmugasundaram, 1989). Plasma lipid levels were expressed as mg/dl and enzymatic activities were expressed as U/L.

2.6. Determination of hepatic oxidative stress parameters

The reactive species (ROS) levels were determined using S1 diluted 1:10 (v/v) in 50 mM Tris–HCl (pH 7.4) and incubated with 10 µl of 2',7'dichlorofluorescein diacetate (DCHF-DA; 1mM) at room temperature for 30 min. The ROS levels were determined by a spectrofluorimetric method, using DCHF-DA assay (Loetchutinat et al., 2005). DCHF-DA is a nonfluorescent compound that easily crosses cell membranes and, in the presence of ROS is rapidly oxidized to its highly fluorescent derivative dichlorofluorescein (DCF). The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) 30 min after the addition of DCHF-DA to the medium. The ROS levels were expressed as nmol DCFA-ox/mg protein.

Non-protein thiols (NPSH) levels were determined by the method of Ellman (Ellman, 1959). S1 was mixed 1:1 (v/v) with 10% trichloroacetic acid and centrifuged 2500 rpm at 4°C

for 10 min. After the centrifugation, the protein pellet was discarded and free–SH groups were determined in the clear supernatant. An aliquot of supernatant was added in 1M potassium phosphate buffer pH 7.4 and 10 mM of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). The colour reaction was measured at 412 nm. NPSH levels were expressed as nmol NPSH/g tissue.

Catalase (CAT) activity was assayed spectrophotometrically by the method of Aebi (Aebi, 1984), which involved monitoring the disappearance of H_2O_2 in the presence of S1 at 240 nm. A sample of S1 was added to 50 mM potassium phosphate buffer pH 7.0 and the enzymatic reaction was initiated by adding H_2O_2. One unit of enzyme was defined as the amount of enzyme required for monitoring the disappearance of H_2O_2. The enzymatic activity was expressed as Unit (U)/mg protein (1 U decomposes 1 mmol H2O2/min at pH 7 at 25°C).
Glutathione peroxidase (GPx) activity was assayed spectrophotometrically by the method of Wendel (Wendel, 1981), through the glutathione/NADPH/glutathione reductase system, by the dismutation of H_2O_2 at 340 nm. S1 was added in the glutathione/NADPH/glutathione reductase system and the enzymatic reaction was initiated by adding H_2O_2. In this assay, the enzyme activity is indirectly measured by means of NADPH decay. H_2O_2 is decomposed, generating glutathione disulfide (GSSG) from glutathione. GSSG is regenerated back to glutathione by glutathione reductase present in the assay medium at the expense of NADPH. The enzymatic activity was expressed as nmol NADPH/min/mg protein.

Superoxide dismutase (SOD) activity was spectrophotometrically determined as described by Misra and Fridovich (Misra and Fridovich, 1972). This method is based on the capacity of SOD in inhibiting autoxidation of epinephrine. The color reaction was measured at 480 nm. The S1 was diluted 1:10 (v/v) for determination of SOD activity in the test day. Aliquots of supernatant were added in a 50 mM Na2CO3 buffer pH 10.3. Enzymatic reaction was started by adding of epinephrine. One unit of enzyme was defined as the amount of

enzyme required to inhibit the rate of epinephrine autoxidation by 50 % at 26 °C. The enzymatic activity was expressed as Units (U)/mg protein.

2.7. Protein determination

Protein concentration was measured by the method of Bradford (Bradford, 1976), using bovine serum albumin as the standard.

2.8. Statistical analysis

The data of $(p\text{-ClPhSe})_2$ were analyzed by two-way ANOVA of variance followed by the Newman – Keuls' test. Main effects are presented only when the first order interaction was non-significant. Comparisons between simvastatin and triton groups were performed by the one-way analysis of variance followed by the Newman-Keul's when necessary. Descriptive statistics data were expressed as the mean(s) ± S.E.M. Probability values less than 0.05 ($P < 0.05$) were considered to be significant.

3. Results

3.1. Effect on activity chamber

The protocol of treatment used in the present study did not cause any alteration in the locomotor and exploratory activities in rats. The statistical analysis revealed that neither crossing, average velocity nor total distance traveled evaluated in the activity chamber were significantly altered in any experimental group (data not shown).

3.2. Effect on body weight gain

Statistical analysis of the body weight gain revealed significant main effects of $(p\text{-ClPhSe})_2$ ($F_{(1,38)} = 101.64$; $p < 0.001$). Acute administration of triton WR-1339 did not alter the body weight gain of animals. The one-way analysis revealed that the administration of simvastatin did not cause any alteration in the body weight gain of rats (Table 1).

3.3. Effect on plasma biochemical parameters

As shown in figure 3A, triton increased TC levels when compared to those of the control group. The two-way analysis of variance of TC data revealed a significant triton and $(p\text{-ClPhSe})_2$ interaction ($F_{(1,38)} = 10.18$; $p < 0.01$). Post hoc analyses indicated that $(p\text{-ClPhSe})_2$ was effective against the increase in TC levels induced by triton injection. The one-way analysis indicated that the administration of simvastatin decreased TC levels increased by triton in rats ($F_{(2,24)} = 20.67$; $p < 0.001$).

As shown in figure 3B, there was an increase in TG levels in response to triton administration. Two-way analysis of variance of TG data exhibited a significant triton and $(p\text{-ClPhSe})_2$ interaction ($F_{(1,37)} = 8.12$; $p < 0.01$). Post hoc analyses indicated that $(p\text{-ClPhSe})_2$ was effective in reducing this parameter increased by triton. The one-way analysis demonstrated that the administration of simvastatin did not reduce TG levels in rats treated with triton.

Figure 3C shows an increase in the CRI index in rats treated with triton. The two-way analysis of variance of CRI data showed a significant triton and $(p\text{-ClPhSe})_2$ interaction ($F_{(1,35)} = 40.22$; $p < 0.001$). Post hoc analyses indicated that $(p\text{-ClPhSe})_2$ protected against the increase of CRI index induced by the triton injection in rats. The one-way analysis revealed that the administration of simvastatin in rats treated with triton decreased the CRI index ($F_{(2.23)} = 18.87$; $p < 0.001$).

The two-way analysis of variance for HDL levels demonstrated a significant triton and $(p\text{-ClPhSe})_2$ interaction ($F_{(1,36)} = 7.30$; $p < 0.05$). Post hoc analyses indicated that triton and $(p\text{-ClPhSe})_2$ did not alter this parameter. By contrast, $(p\text{-ClPhSe})_2$ increased the HDL levels in animals of the triton group. The one-way analysis demonstrated that the administration of simvastatin did not cause any alteration in the HDL levels in rats (Table 2).

Triton increased non-HDL levels in rats. The two-way analysis of variance for non-HDL levels demonstrated a significant triton and $(p\text{-ClPhSe})_2$ interaction ($F_{(1,36)} = 15.46$; $p < 0.001$). Post hoc analyses revealed that $(p\text{-ClPhSe})_2$ was effective in preventing the increase of non-HDL levels caused by triton. The one-way analysis indicated that the administration of simvastatin decreased this parameter in rats ($F_{(2.23)} = 32.09$; $p < 0.001$) (Table 2).

As depicted in figure 3D, triton increased the activity of ALT. Two-way analysis of variance showed a significant triton and $(p\text{-ClPhSe})_2$ interaction ($F_{(1,37)} = 10.72$; $p < 0.01$). Post hoc analyses demonstrated that $(p\text{-ClPhSe})_2$ was effective against the increase of ALT activity caused by the triton injection in rats. The one-way analyses indicated that the simvastatin protected against the increase in ALT activity ($F_{(2,24)} = 7.65$; $p < 0.05$).

Regarding the AST activity, there was an increase in this enzyme activity in rats treated with triton (Figure 3E). Two-way ANOVA revealed a significant triton and $(p\text{-ClPhSe})_2$ interaction ($F_{(1,38)} = 5.02$; $p < 0.05$). Post hoc analyses exhibited that $(p\text{-ClPhSe})_2$ was effective in reducing this parameter. The one-way analysis showed that the administration of simvastatin to rats decreased the AST activity ($F_{(2,24)} = 5.37$; $p < 0.05$).

3.4. Effect on hepatic oxidative stress parameters

Triton increased the ROS levels in livers of rats when compared with those of the control group. Statistical analysis of ROS levels demonstrated that there was a main effect of triton ($F_{(1,27)} = 22.82$; $p < 0.001$). $(p\text{-ClPhSe})_2$ did not protect against the increase of ROS

levels induced by triton. According to one-way analyses, ROS levels increased in livers of rats treated with simvastatin (Table 3).

The two-way analysis of NPSH levels indicated a significant triton and $(p\text{-ClPhSe})_2$ interaction ($F_{(1,34)} = 5.56$; $p < 0.05$). According to post hoc analyses, hepatic NPSH levels increased in rats treated with triton and $(p\text{-ClPhSe})_2$. One-way analyses revealed that NPSH levels were not altered in rats in the simvastatin group (Table 3).

The two-way analysis of CAT activity showed a significant triton and $(p\text{-ClPhSe})_2$ interaction ($F_{(1,18)} = 10.78$; $p < 0.01$). Post hoc analyses indicated that CAT activity remained unaltered in all experimental groups. The one-way analyses revealed that rats treated with simvastatin had CAT activity similar to that of the control group (Table 3).

Two-way analysis of the GPx and SOD activities indicated that there was no significant triton and $(p\text{-ClPhSe})_2$ interaction. Moreover, the one-way analyses revealed that simvastatin had no effect on these enzymes (Table 3).

DISCUSSION

The current study aimed at investigating the antihyperlipidemic and hepatoprotective effects of $(p\text{-ClPhSe})_2$ in triton WR-1339 induced hyperlipidemic rats. Triton acts as a surfactant and causes structural modifications in circulatory lipoproteins, suppresses the action of lipases and as a consequence blocks the uptake of circulating lipids by extra hepatic tissues, resulting in increased blood lipid concentration (Schurr et al., 1972). Consistent with previous findings, in our study the plasma lipid levels were altered after a triton injection (Takahashi et al., 2003, Kim et al., 2002, Saravanan et al., 2011). In this study, animals of triton group showed an increase in TC, TG, non-HDL levels and CRI index. Similar to other

work (Jamshed and Gilani, 2014), the HDL levels showed no reduction after triton injection in this study.

As reported in a recent study (Bortolatto et al., 2015), our results showed that the treatment with $(p\text{-ClPhSe})_2$ prevented the gain of body weight (table 1). This effect is relevant in view of the fact that weight loss protects against liver chemistries, steatosis, necro-inflammatory changes and fibrosis (Huang et al., 2005, Petersen et al., 2005, Suzuki et al., 2005). Furthermore, $(p\text{-ClPhSe})_2$ had a beneficial effect on the lipid levels by reducing plasma TC, TG, non-HDL and CRI as well as increasing the HDL levels in animals treated with triton WR-1339. Besides, our data also showed that treatment with this organoselenium compound did not trigger impairments on locomotor and exploratory activities, thus our results indicate that $(p\text{-ClPhSe})_2$ showed a hypolipidemic potential, without changing locomotor activity. As expected, simvastatin decreased TC levels in rats treated with triton. The main mechanism of action of simvastatin is the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase, a rate-limiting enzyme of cholesterol synthesis (Endo, 1992, Tobert, 2003). While simvastatin action was exclusively to reduce TC levels, $(p\text{-ClPhSe})_2$ compound was effective to reduce not only TC levels but also TG levels.

The liver has a central role in the metabolism, transport and clearance of xenobiotics and is therefore highly susceptible to chemical-induced toxicity. The enzymes AST and ALT are well-known biomarkers of hepatic damage (Recknagel et al., 1989). Our findings demonstrated that the plasma activities of both enzymes were higher in livers of rats treated with triton, which are consistent with the results of a previous study (Anandhi et al., 2013). The animals treated with $(p\text{-ClPhSe})_2$ showed normal AST and ALT activities, suggesting a hepatoprotective activity of this selenium compound against hepatotoxicity caused by triton. Similarly, simvastatin also reduced the activities of ALT and AST in the triton-treated group. Indeed, statins have shown strong hepato and vasoprotective effects in livers from healthy,

cirrhotic and obese animals (Russo et al., 2012, Abraldes et al., 2007, Ajamieh et al., 2012, Trebicka et al., 2007). Furthermore, simvastatin ameliorates hepatic microcirculation and endothelial dysfunction in steatotic cold-stored and warm-reperfused livers in rats (Gracia-Sancho et al., 2013).

The fatty liver is among the biggest liver damage mediated by oxidative stress (Muriel, 2009). Oxidative stress is one of the main causes of liver injury that depletes the antioxidant enzyme sources and decreases the ability of cells in functioning against injury (Muriel, 2009). As shown in table 3, the triton treated animals showed an increase in ROS levels. Accordingly, the relationship between increase ROS production and hepatocyte damage has been reported (Jaeschke et al., 2012). Taking into account that the liver plays a central role in the maintenance of systemic lipid homeostasis, it is especially susceptible to damage by ROS (Hamelet et al., 2007). Furthermore, it was reported that ROS levels in hypercholesterolemia were higher than in the normal state (Stokes et al., 2002).

A number of studies have reported the beneficial effects of organoselenium compounds against pathological conditions associated with oxidative stress (inflammation, diabetes, neurotoxicity and hepatotoxicity) (Nogueira and Rocha, 2011). In the present study, (p-ClPhSe)2 did not protect against the increase of ROS levels, but was effective in increasing NPHS levels in animals treated with triton. By contrast, simvastatin protected against the increase of ROS levels induced by triton, but did not alter NPSH levels. Indeed, simvastatin has also been shown to be a potent antioxidant in vivo (Davignon et al., 2004, Tomas et al., 2000, Moon et al., 2011).

In conclusion, our findings suggest that the administration of $(p\text{-ClPhSe})_2$ at a dose of 10 mg/kg for seven days to rats resulted in a significant decrease in plasma lipid levels and that $(p\text{-ClPhSe})_2$ was effective in normalizing ALT and AST activities. It is therefore reasonable to assume that $(p\text{-ClPhSe})_2$ can help in the development of new therapeutic

approaches to reduce damage caused by fat excess or toxic agents and hyperlipidemia. However, further studies are needed to elucidate the mechanism of action by which (p-ClPhSe)$_2$ has hypolipidemic and hepatoprotective effects.

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DECLARATION OF INTEREST

The authors declare that there are no conflicts of interest.

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Legends

Figure 1. Chemical structure of 4,4'-dichlorodiphenyl diselenide [(p-ClPhSe)2].

Figure 2. Experimental protocol

Figure 3. Effect of (p-ClPhSe)2, Simvastatin and Triton-WR 1339 on plasma biochemical parameters. (A) Total cholesterol levels; (B) Triglycerides levels; (C) CRI index; (D) aspartate aminotransferase; (E) alanine aminotransferase. Each column represents the mean ± SEM of 8-10 rats in each group. [Sim] indicates animals treated with Simvastatin. Asterisks denote the significance levels when compared to the control group (Two-way ANOVA followed by the Newman-Keuls' test) (**) $p < 0.01$. Sharps mean the significance levels when compared with the Triton group (Two-way ANOVA followed by the Newman-Keuls' test) (##) $p < 0.01$. Arrobas imply the significance.

Figure 1.

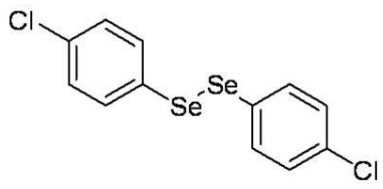


Figure 2.

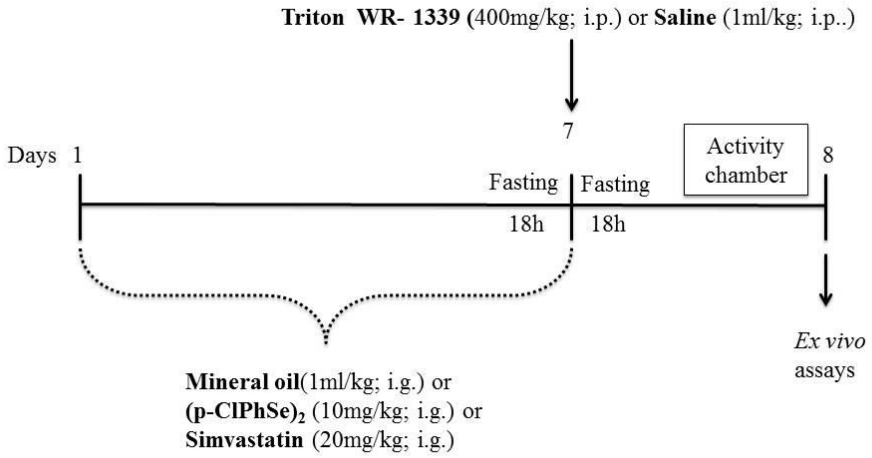
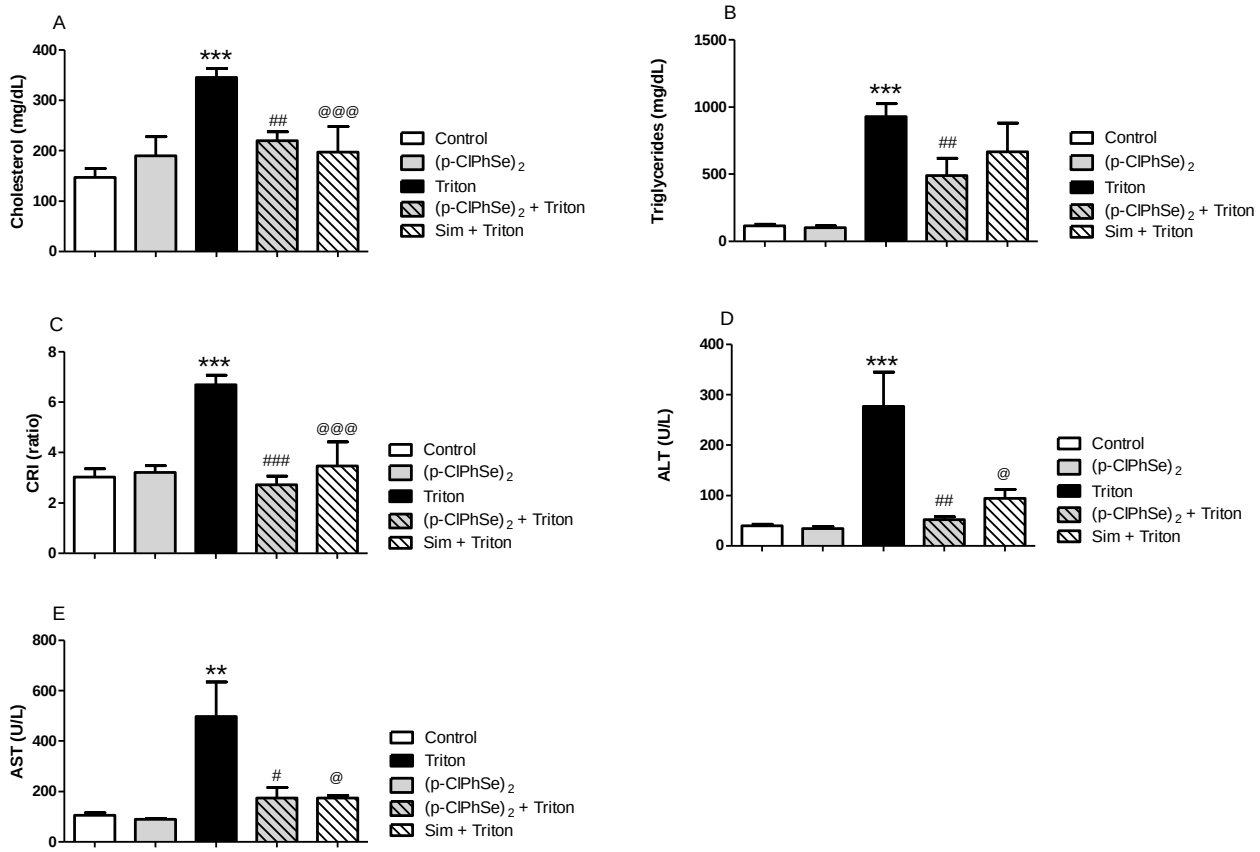

Triton WR- 1339 (400mg/kg; i.p.) or Saline (1ml/kg; i.p..)
7
Days 1
Activity chamber
8
Fasting
18h
Fasting
18h
Ex vivo assays
Mineral oil(1ml/kg; i.g.) or
(p-ClPhSe)2 (10mg/kg; i.g.) or
Simvastatin (20mg/kg; i.g.)

Figure 3.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Triton
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Triglycerides (mg/dL)
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Triton
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Table 1. Body weight gain of rats exposed to triton WR-1339 and treated with simvastatin or (*p*-ClPhSe)$_2$.

Groups	Body weight gain[a]
Control	3.77 ± 2.39
Triton	1.60 ± 2.29
(p-ClPhSe)$_2$	-24.08 ± 2.63***
(p-ClPhSe)$_2$ + Triton	-22.70 ± 2.81***
Sim + Triton	0.60 ± 2.48

Data are reported as the mean ± SEM of 9-12 rats in each group. [Sim] indicates animals treated with simvastatin. Asterisks denote the significance levels when compared to the control group (Two-way ANOVA followed by the Newman-Keuls' test) (***) $p < 0.001$.[a] g/kg.

Table 2. Plasma biochemical parameters of rats exposed to triton-WR 1339 and treated with simvastatin or (*p*-ClPhSe)$_2$.

Groups	HDL[a]	non-HDL[a]
Control	48.50 ± 2.41	98.70 ± 16.68
Triton	53.89 ± 3.03	276.70 ± 28.23**
(p-ClPhSe)$_2$	48.21 ± 2.59	142.10 ± 38.58
(p-ClPhSe)$_2$ + Triton	87.33 ± 12.78$^{\#\#\#}$	129.20 ± 18.28$^{\#\#\#}$
Sim + Triton	55.25 ± 12.92	118.60 ± 36.17$^{@@@}$

Data are reported as the mean ± SEM of 9-12 rats in each group. [Sim] indicates animals treated with simvastatin. Asterisks denote the significance levels when compared to the control group (Two-way ANOVA followed by the Newman-Keuls' test) (**) $p < 0.01$. Sharps mean the significance levels when compared to the Triton group (Two-way ANOVA followed by the Newman-Keuls' test) ($^{\#\#\#}$) $p < 0.001$. Arrobas imply the significance levels when compared to the Triton group (One-way ANOVA followed by the Newman-Keuls' test) ($^{@@@}$) $p < 0.001$.[a] mg/dl.

Table 3. Parameters of oxidative stress in liver of rats exposed to Triton-WR 1339 and treated with simvastatin or $(p\text{-ClPhSe})_2$.

Groups	ROS	NPSH	GPx	SOD	CAT
Control	211.30 ± 25.05	6.25 ± 0.49	100.70 ± 3.44	25.49 ± 1.46	20.35 ± 0.91
Triton	247.00 ± 25.35***	4.89 ± 0.38	116.40 ± 5.97	24.61 ± 2.36	20.07 ± 0.24
$(p\text{-ClPhSe})_2$	213.70 ± 22.10	7.73 ± 0.55	95.72 ± 7.19	22.97 ± 1.32	27.24 ± 1.71
$(p\text{-ClPhSe})_2$ + Triton	253.30 ± 25.44	8.88 ± 0.68$^{\#}$	99.34 ± 5.06	25.68 ± 0.42	20.39 ± 0.84
Sim + Triton	175.60 ± 14.69$^{@@}$	5.31 ± 0.58	102.4 ± 14.19	24.68 ± 2.38	19.10 ± 1.78

Data are reported as the mean ± SEM of 5-7 rats in each group. [Sim] indicates animals treated with simvastatin. Asterisks denote the significance levels when compared to the control group (Two-way ANOVA followed by the Newman-Keuls' test) (***) $p < 0.001$. Sharps mean the significance levels when compared to the Triton group (Two-way ANOVA followed by the Newman-Keuls' test) ($^{\#}$) $p < 0.05$. Reactive Oxygen Species (ROS) nmol DCFA-ox/mg protein, non-protein thiols (NPSH) nmol NPSH/g tissue, glutathione peroxidase (GPx) nmol NADPH/min/mg protein, superoxide dismutase (SOD) U/mg protein, catalase (CAT) U/mg protein/min.

4 CONCLUSÃO

Os resultados apresentados nesta dissertação permitem concluir que:

O tratamento com $(p\text{-ClPhSe})_2$ foi eficaz em reverter os parâmetros bioquímicos relacionados à hiperlipidemia e toxicidade hepática induzidos pela administração de triton WR-1339 em ratos.

A administração do $(p\text{-ClPhSe})_2$ não foi eficaz em proteger do aumento de EROS induzido por triton WR-1339.

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