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

EFEITOS FARMACOLÓGICOS DO DISSELENETO DE DIFENILA EM MODELOS DE TOXICIDADE INDUZIDA POR ORGANOFOSFORADOS EM RATOS

TESE DE DOUTORADO

Carmine Inês Acker

Santa Maria, RS, Brasil

2012

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por

Carmine Inês Acker

Tese 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 parcial para a obtenção do grau de **Doutor em Bioquímica Toxicológica**.

Orientadora: Profª Drª Cristina Wayne Nogueira

Santa Maria, RS, Brasil

2012

Universidade Federal de Santa Maria
Centro de Ciências Naturais e Exatas
Programa de Pós-Graduação em Ciências Biológicas:
Bioquímica Toxicológica

A Comissão Examinadora, abaixo assinada, aprova a Tese de Doutorado

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elaborada por

Carmine Inês Acker

como requisito parcial para obtenção do grau de
Doutor em Bioquímica Toxicológica

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Santa Maria, 10 de agosto de 2012

AGRADECIMENTOS

Aos meus pais, pelo amor, pela dedicação, pelo incentivo e principalmente por me apoiarem em todos os momentos.

À minha irmã, Patrícia, pela amizade, pelo incentivo, pelo exemplo de força, e por sempre estar presente, mesmo que distante.

Ao Helton, pelo amor, carinho, amizade e compreensão.

À professora Cristina e ao professor Gilson, pelo exemplo de profissionalismo, pela orientação, pelos ensinamentos e pela amizade.

Aos colegas de laboratório: Ana Cristina, Ethel, Bibiana, Simone, Juliana, Marina, Danieli, Marlon, César, Cristiani, Gláubia, Michael, Pietro, Carla, Suélen, Ana Paula, Suelen, Marcel, Tuane, Suzan, Caroline e Franciele. Obrigada por toda a ajuda e pela amizade.

Aos colegas do laboratório do professor Gilson.

À todos os meus amigos, em especial às minhas queridas amigas Sinara e Margiani, por todo o apoio, pela amizade e simplesmente por fazerem parte da minha vida.

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Bioquímica Toxicológica.

Aos professores do Programa de Pós-graduação em Bioquímica Toxicológica.

À CAPES, pelo auxílio financeiro durante a realização deste trabalho.

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

EFEITOS FARMACOLÓGICOS DO DISSELENETO DE DIFENILA EM MODELOS DE TOXICIDADE INDUZIDA POR ORGANOFOSFORADOS EM RATOS

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LOCAL E DATA DA DEFESA: Santa Maria, agosto de 2012

Os agrotóxicos são substâncias empregadas nas áreas agrícolas e em programas de saúde pública, para o controle de pragas e vetores que transmitem doenças. Dentre os agrotóxicos, os inseticidas organofosforados (OFs) são considerados os mais tóxicos aos vertebrados. O disseleneto de difenila [$(PhSe)_2$] é um composto orgânico de selênio para o qual já foram descritas diversas propriedades farmacológicas, entre elas a atividade antioxidante. Dessa forma, este trabalho teve como objetivos avaliar os efeitos farmacológicos do $(PhSe)_2$ em modelos de toxicidade aguda induzida por clorpirifós (CPF) e acefato (AC) em ratos, bem como, avaliar os efeitos hiperglicêmico e hiperlipidêmico do CPF, os quais não estão descritos na literatura. No primeiro protocolo experimental (**artigo 1**), avaliou-se o efeito do $(PhSe)_2$ na toxicidade hepática e hematológica induzida por CPF em ratos. Os animais foram pré-tratados com $(PhSe)_2$ (5 mg/kg) pela via intragástrica (p.o.) uma vez ao dia durante 7 dias. No 8º e 9º dias o $(PhSe)_2$ (5 mg/kg; p.o.) foi administrado 30 min antes da administração subcutânea (s.c.) de CPF (50 mg/kg). Os animais foram mortos vinte e quatro horas após a última administração de CPF. A atividade das enzimas aspartato aminotransferase (AST), alanina aminotransferase (ALT) e lactato desidrogenase (LDH) foram determinadas no plasma dos ratos. Os níveis de peroxidação lipídica, carbonilação de proteínas e tióis não-protéicos (SHNP), bem como a atividade das enzimas catalase (CAT), superóxido dismutase (SOD), glutationa peroxidase (GPx), glutationa redutase (GR) e glutationa S-transferase (GST) foram determinados no fígado dos ratos. Os parâmetros hematológicos também foram analisados. O CPF causou aumento da atividade das enzimas AST, ALT e LDH, aumento dos níveis de peroxidação lipídica e carbonilação de proteínas, diminuição dos níveis de SHNP e inibição das enzimas CAT, GPx, SOD e GST. Além disso, a exposição ao CPF causou toxicidade hematológica, evidenciada principalmente pela diminuição dos níveis de leucócitos totais. O $(PhSe)_2$ protegeu contra os efeitos tóxicos induzidos pelo CPF em ratos. Além disso, o $(PhSe)_2$ aumentou *per se* os níveis de SHNP e a atividade da GST no fígado dos ratos. No segundo protocolo experimental (**artigo 2**), investigou-se o efeito do $(PhSe)_2$ nos distúrbios metabólicos induzidos por AC em ratos. O $(PhSe)_2$ (10 ou 30 mg/kg; p.o.) foi administrado aos animais 1 hora antes da administração de AC (140 mg/kg; p.o.). Os animais foram mortos duas horas após a administração de AC. Os níveis de glicose e corticosterona bem como o perfil lipídico foram determinados no plasma dos ratos. Os fatores de risco cardiovascular e o índice aterogênico foram calculados. Os níveis de glicogênio bem como a atividade das enzimas tirosina aminotransferase (TAT) e glicose-6-fosfatase (G6Pase) foram analisados no fígado dos ratos. A atividade da acetilcolinesterase (AChE) cerebral também foi determinada. O AC causou aumento dos níveis de glicose, corticosterona e triglicerídios (TG), aumento da atividade das enzimas TAT e G6Pase e inibição da AChE. O fator de risco cardiovascular [(TG/lipoproteína de alta densidade (HDL)] aumentou nos ratos expostos ao AC. O $(PhSe)_2$ atenuou essas alterações,

exceto para o aumento dos níveis de corticosterona e para a inibição da AChE. No terceiro protocolo experimental (**artigo 3**), investigou-se o efeito hiperglicêmico e hiperlipidêmico do CPF em ratos. Também foram estudados os mecanismos envolvidos no efeito hiperglicêmico do CPF. O CPF foi administrado uma única vez na dose de 50 mg/kg, s.c.. Os animais foram mortos em diferentes tempos após a administração de CPF (2, 4, 8, 12 e 24 horas). Os níveis de glicose e corticosterona bem como o perfil lipídico e a atividade da paraoxonase-1 (PON-1) foram determinados no plasma dos ratos. Os fatores de risco cardiovascular e o índice aterogênico foram calculados. Os níveis de glicogênio bem como a atividade das enzimas TAT e G6Pase foram analisados no fígado dos ratos. A atividade da AChE cerebral também foi determinada. O CPF causou aumento dos níveis de glicose, glicogênio, corticosterona, TG e lipoproteína de baixa densidade (LDL), aumento da atividade das enzimas TAT e G6Pase, diminuição dos níveis de HDL e da atividade da PON-1 e inibição da atividade da AChE. Os fatores de risco cardiovascular e o índice aterogênico aumentaram nos animais expostos ao CPF. Os resultados do presente trabalho demonstraram que o $(PhSe)_2$ protegeu contra a toxicidade induzida por CPF e AC em ratos. A exposição ao CPF causou hiperglicemia e hiperlipidemia em ratos. A ativação da via da gliconeogênese está envolvida no efeito hiperglicêmico causado pelo CPF. Considerando-se que a exposição aos OFs é cada vez mais freqüente e que é a causa de diversas doenças, os resultados deste trabalho são de grande importância, uma vez que o $(PhSe)_2$ pode representar uma alternativa para atenuar a toxicidade causada pelos OFs.

Palavras-chave: Agrotóxicos, Organofosforados, Clorpirifós, Acefato, Selênio, Disseleneto de Difenila.

ABSTRACT

Thesis of Doctor's Degree
Federal University of Santa Maria, RS, Brazil

PHARMACOLOGICAL EFFECTS OF DIPHENYL DISELENIDE AGAINST ORGANOPHOSPHATE-INDUCED MODELS OF TOXICITY IN RATS

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ADVISOR: Cristina Wayne Nogueira
DATE AND PLACE OF THE DEFENSE: Santa Maria, august, 2012

Pesticides are substances used in agricultural areas and public health programs to control pests and disease vectors. Among pesticides, organophosphates (OPs) are considered the most toxic to vertebrates. Diphenyl diselenide [$(PhSe)_2$] is an organoselenium compound that presents pharmacological activities, among that the antioxidant effect. Therefore, the aim of this study was to evaluate the pharmacological effects of $(PhSe)_2$ in acute models of toxicity induced by chlorpyrifos (CPF) and acephate (AC) in rats, as well as to investigate the hyperglycemic and hyperlipidemic effects of CPF which has not been described. In the first experimental protocol (**article 1**), the effect of $(PhSe)_2$ on hepatic and hematological toxicity induced by CPF in rats was evaluated. The animals were pre-treated by intragastric route (p.o.) with $(PhSe)_2$ (5 mg/kg) once a day for 7 days. On the 8^{th} and 9^{th} days, $(PhSe)_2$ (5 mg/kg; p.o.) was administered to rats 30 min prior to subcutaneous (s.c.) injection of CPF (50 mg/kg). Twenty-four hours after the last CPF injection, rats were killed. The aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activities were determined in plasma of rats. Lipid peroxidation, protein carbonyl and non-protein thiol (NPSH) levels as well as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and gluthatione S-transferase (GST) activities were determined in livers of rats. Hematologic parameters were also assayed. CPF caused an increase in AST, ALT and LDH activities, an increase in lipid peroxidation and protein carbonyl levels, a decrease in NPSH levels and an inhibition of CAT, GPx, SOD and GST activities. In addition, CPF exposure caused hematologic toxicity, evidenced mainly by a decrease in total leukocytes levels. $(PhSe)_2$ protected against toxic effects induced by CPF in rats. Moreover, $(PhSe)_2$ increased *per se* NPSH levels and GST activity in livers of rats. In the second experimental protocol (**article 2**), the effect of $(PhSe)_2$ on metabolic disorders induced by AC in rats was investigated. $(PhSe)_2$ (10 or 30 mg/kg; p.o.) was administered to rats 1 hour prior to AC administration (140 mg/kg; p.o.). Two hours after AC administration, rats were killed. Glucose and corticosterone levels as well as the lipid status were determined in plasma of rats. Cardiovascular risk factor and the atherogenic index were calculated. Glycogen levels as well as tyrosine aminotransferase (TAT) and glucose-6-phosphatase (G6Pase) activities were determined in livers of rats. Cerebral acetylcholinesterase (AChE) activity was assayed. AC induced an increase in glucose, corticosterone and triglycerides (TG) levels, an increase in TAT and G6Pase activities and an inhibition of AChE activity. The cardiovascular risk factor [(TG/ high density lipoprotein (HDL)] was increased in AC exposed rats. $(PhSe)_2$ attenuated these alterations, except for the

increase of corticosterone levels and AChE activity inhibition. In the third experimental protocol (**article 3**), the hyperglycemic and hyperlipidemic effects of CPF in rats were investigated. The mechanisms involved in hyperglycemia induced by CPF were also studied. CPF was administered once to rats at the dose of 50 mg/kg, s.c. Animals were killed at 2, 4, 8, 12 e 24 hours after CPF administration. Glucose and corticosterone levels as well as lipid status and paraoxonase-1 (PON-1) activity were determined in plasma of rats. Cardiovascular risk factors and the atherogenic index were calculated. Glycogen levels as well as TAT and G6Pase activities were determined in livers of rats. Cerebral AChE activity was assayed. CPF caused an increase in glucose, glycogen, corticosterone, TG and low density lipoprotein (LDL) levels, an increase in TAT and G6Pase activities, a decrease in HDL levels and PON-1 activity and AChE activity inhibition. The cardiovascular risk factors and atherogenic index were increased in CPF exposed rats. The results of the present study demonstrated that $(PhSe)_2$ protected against toxic effects induced by CPF and AC in rats. CPF exposure caused hyperglycemia and hyperlipidemia in rats. The gluconeogenesis pathway activation is involved in the hyperglycemic effect caused by CPF. Considering that the crescent use of OPs worldwide has been the cause of many severe human poisoning cases, the results of the present work are of great importance, since that $(PhSe)_2$ may represent an alternative to alleviate the OPs-induced toxicity.

Keywords: Pesticides, Organophosphate, Chlorpyrifos, Acephate, Selenium, Diphenyl diselenide.

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LISTA DE ABREVIATURAS

δ-ALA-D – δ-Aminolevulinato desidratase

AC – Acefato

ACh – Acetilcolina

AChE – Acetilcolinesterase

ACTH – Hormônio adrenocorticotrópico

AGEs – Produtos finais de glicação avançada

ALT – Alanina aminotransferase

AST – Aspartato aminotransferase

CAT – Catalase

CPF – Clorpirifós

CRH – Hormônio corticotropina

CT – Colesterol Total

DNA – Ácido desoxirribonucléico

EROs – Espécies reativas de oxigênio

G6Pase – Glicose-6-fosfatase

GPx – Glutationa peroxidase

GST – Glutationa S-transferase

HDL – Lipoproteína de alta densidade

HPA – Hipotálamo-pituitária-adrenal

IA – Índice Aterogênico

LDH – Lactato desidrogenase

LDL – Lipoproteína de baixa densidade

OFs – Inseticidas Organofosforados

$(PhSe)_2$ – Disseleneto de difenila

PON-1 – Paraoxonase-1

RAGEs – Receptores de AGEs

SHNP – Tióis não-protéicos

Sinitox – Sistema Nacional de Informações Tóxico-Farmacológicas

SOD – Superóxido dismutase

TAT – Tirosina Aminotransferase

TG – Triglicerídios

SUMÁRIO

1 INTRODUÇÃO

Os agrotóxicos são substâncias empregadas nas áreas agrícolas e em programas de saúde pública, para o controle de pragas e vetores que transmitem doenças. Atualmente, centenas de ingredientes ativos e milhares de formulações estão disponíveis no mercado mundial, sendo que milhões de toneladas de agrotóxicos são fabricados anualmente (Soltaninejad e Abdollahi, 2009). Segundo dados da Organização Mundial da Saúde, ocorrem cerca de 3 a 5 milhões de casos de intoxicação por agrotóxicos todos os anos, resultando em centenas de mortes, principalmente entre trabalhadores agrícolas. A maioria destas intoxicações ocorre em países em desenvolvimento, onde a falta de higiene, informação ou controle adequado têm criado uma predisposição aos efeitos tóxicos desses compostos.

No Brasil, os últimos dados disponíveis pelo Sistema Nacional de Informações Tóxico-Farmacológicas (Sinitox) mostram que os agrotóxicos, divididos em quatro categorias (agrotóxicos/uso agrícola, agrotóxicos/uso doméstico, raticidas e produtos veterinários) são a 2º maior causa de intoxicação em humanos, com 11.484 casos em 2009, ficando atrás apenas dos medicamentos. As regiões brasileiras que mais registraram casos de intoxicação são a região sudeste com 5.151 casos (45%) e a região sul com 2.708 (24%) (Sinitox, 2009).

Dentre os agrotóxicos, os inseticidas organofosforados (OFs) são considerados os mais tóxicos aos vertebrados (Shadnia *et al*., 2005; Rahimi *et al*., 2006). Os OFs foram sintetizados pela primeira vez na Alemanha antes da Segunda Guerra Mundial e hoje constituem uma das classes de agrotóxicos mais utilizados no mundo. A sua aplicação cada vez mais intensa têm resultado em uma poluição ambiental e também no aumento do número de casos de intoxicações, tanto agudas quanto crônicas (Costa, 2006). São exemplos de OFs: azinfós etílico, clorpirifós (CPF), acefato (AC), diclorvos, dimetoato, diazinon, fenitrotion, fention, fosfamidon, malation, metamidofós, monocrotofós, paration metílico. Estes compostos são facilmente hidrolisados e altamente lipossolúveis, com alto coeficiente de partição óleo/água (Soltaninejad e Abdollahi, 2009).

A absorção dos OFs pelo organismo humano ocorre pelas vias dérmica, respiratória e digestiva, e muitas vezes essa absorção é favorecida pelos solventes presentes na formulação. As intoxicações podem ocorrer por contato direto com os OFs, no preparo, na aplicação ou em qualquer tipo de manuseio desses compostos. Podem ainda ocorrer por contato indireto, pela contaminação da água, do ar, do solo e dos alimentos. Também há casos de intoxicações

acidentais, sobretudo em crianças, e intencionais (homicídios e suicídios) (Alonzo e Corrêa, 2002).

Os OFs têm como principal alvo a enzima acetilcolinesterase (AChE) que hidroliza o neurotransmissor acetilcolina (ACh). Os principais efeitos clínicos observados em intoxicações agudas com esses compostos são lacrimação, salivação, vômitos, diarréia, dores de cabeça, convulsões. Esses efeitos envolvem a inibição irreversível da atividade da AChE no sangue e no sistema nervoso, resultando no acúmulo de ACh e ativação dos receptores muscarínicos e nicotínicos, o que pode levar à morte (Savolainen, 2001; Aygun *et al*., 2007).

Embora o principal alvo de toxicidade dos OFs seja a inibição da atividade da AChE no sistema nervoso central, muitos autores têm demonstrado outros efeitos tóxicos, após exposições agudas e crônicas. Já foram demonstrados efeitos tóxicos em diversos sistemas e órgãos, tais como, fígado, rim, músculo, sistema imune, sistema hematológico e outros (Abdollahi *et al*., 2004a; Teimouri *et al*., 2006; Possamai *et al*., 2007). Alguns desses efeitos envolvem o distúrbio de processos redox com alterações na atividade de enzimas antioxidantes e aumento dos níveis de peroxidação lipídica (Sharma *et al*., 2005; Fortunato *et al*., 2006). Dessa forma, a indução de estresse oxidativo tem sido considerada um dos mecanismos de toxicidade dos OFs (Lukaszewicz-Hussain, 2010). O estresse oxidativo é definido como um desequilíbrio entre a produção de espécies reativas e a sua remoção pelas defesas antioxidantes. As espécies reativas produzidas em excesso causam danos a lipídios, proteínas e DNA e, conseqüentemente o desenvolvimento de diversas doenças (Halliwell, 2011).

Outro efeito tóxico que vem sendo descrito nos últimos anos é o distúrbio da homeostase da glicose observado após a exposição à OFs. Já foi demonstrado que OFs como AC, malation e monocrotofós causam hiperglicemia temporária após a administração aguda em animais experimentais (Lasram *et al*., 2008; Joshi e Rajini, 2009; 2012). No entanto, diversos mecanismos parecem estar envolvidos na hiperglicemia induzida pelos OFs. Alguns autores relatam o envolvimento da ativação de enzimas da gliconeogênese (fosfoenolpiruvato carboxiquinase, glicose-6-fosfatase (G6Pase)) e glicogenólise (glicogênio fosforilase), bem como a inibição de enzimas glicolíticas (hexoquinase, fosfofrutoquinase) na hiperglicemia induzida por OFs (Abdollahi *et al*., 2004b; Rezg *et al*., 2007).

Recentemente Joshi e Rajini (2009) descreveram que os OFs podem interferir no funcionamento do eixo hipotálamo-pituitária-adrenal (HPA), o qual regula o processo de síntese e secreção de glicocorticóides pela glândula adrenal. A liberação do hormônio corticotropina (CRH) pelo hipotálamo estimula a glândula pituitária a liberar o hormônio

adrenocorticotrópico (ACTH), que por sua vez estimula a síntese e secreção de glicocorticóides pela glândula adrenal. Os hormônios glicocorticóides liberados pela glândula adrenal aumentam a produção de glicose hepática através do aumento da atividade de enzimas da gliconeogênese. Recentemente foi demonstrado que os OFs causam hiperglicemia através do aumento dos níveis de corticosterona no plasma e conseqüentemente da atividade da tirosina aminotransferase (TAT), enzima que participa da degradação de aminoácidos e, portanto, estimula a gliconeogênese (Joshi e Rajini, 2009; 2012). A inibição da AChE no sistema nervoso central pelos OFs parece estar envolvida na indução de hipercorticosteronemia, uma vez que o excesso de ACh estimula o hipotálamo a liberar o CRH que irá ativar o eixo HPA e conseqüentemente estimular a gliconeogênese (Bugajski *et al*., 2001).

Embora haja poucos dados na literatura com relação ao efeito dos OFs sobre o metabolismo de lipídios, a hiperlipidemia também tem sido descrita como um dos efeitos adversos da exposição a esses compostos. Lasram e colaboradores (2009) demonstraram que a exposição aguda ao malation em ratos causou um aumento dos níveis de triglicerídios (TG) e de lipoproteínas de baixa densidade (LDL). Foi demonstrado ainda que esse inseticida aumentou os fatores de risco cardiovascular [TG/lipoproteína de alta densidade (HDL) e colesterol total (CT)/HDL] e o índice aterogênico (IA) [(CT-HDL)/HDL]. De maneira similar, a administração de propetamfós em ratos levou ao aumento dos níveis de TG (Çetin *et al*., 2010). No entanto, ainda não há dados na literatura mostrando quais são os mecanismos envolvidos na hiperlipidemia induzida pelos OFs.

O CPF, O,O'-dietil O-(3,5,6-tricloro-2-piridila) fosforotioato, é um dos cinco inseticidas OFs mais comercializados no mundo com mais de 900 formulações diferentes. Esse inseticida é utilizado na agricultura para o controle de pragas em diversas culturas e também é aplicado em áreas domésticas. Pertence à classe toxicológica II (muito tóxico), apresentando dose letal de 50% dos animais (DL_{50}) de 135 mg/kg quando administrado pela via oral em ratos (Eaton *et al*., 2008). O CPF, uma vez absorvido, é metabolicamente ativado a CPF-oxon, forma mais tóxica desse inseticida, responsável pelos efeitos tóxicos em mamíferos (Kousba *et al*., 2004). Essa metabolização ocorre no fígado através de uma reação de dessulfuração oxidativa catalisada pelas enzimas do citocromo P450. O CPF-oxon formado pode ser metabolizado por esterases hepáticas e extra-hepáticas como a Paraoxonase-1 (PON-1), enzima que participa, portanto, da detoxificação do CPF-oxon (Busby-Hjerpe *et al*., 2010). A biotransformação do CPF está demonstrada na Figura 1.

Diversos efeitos tóxicos têm sido relatados após exposições agudas e crônicas ao CPF em animais experimentais, tais como alterações imunológicas (Thrasher *et al*., 1993), hepatotoxicidade e nefrotoxicidade (Verma e Srivastava, 2003), teratogenicidade (Tian *et al*., 2005), genotoxicidade (Mehta *et al*., 2008), entre outras. No entanto, não há relatos na literatura sobre os seus efeitos no metabolismo de carboidratos e lipídios.

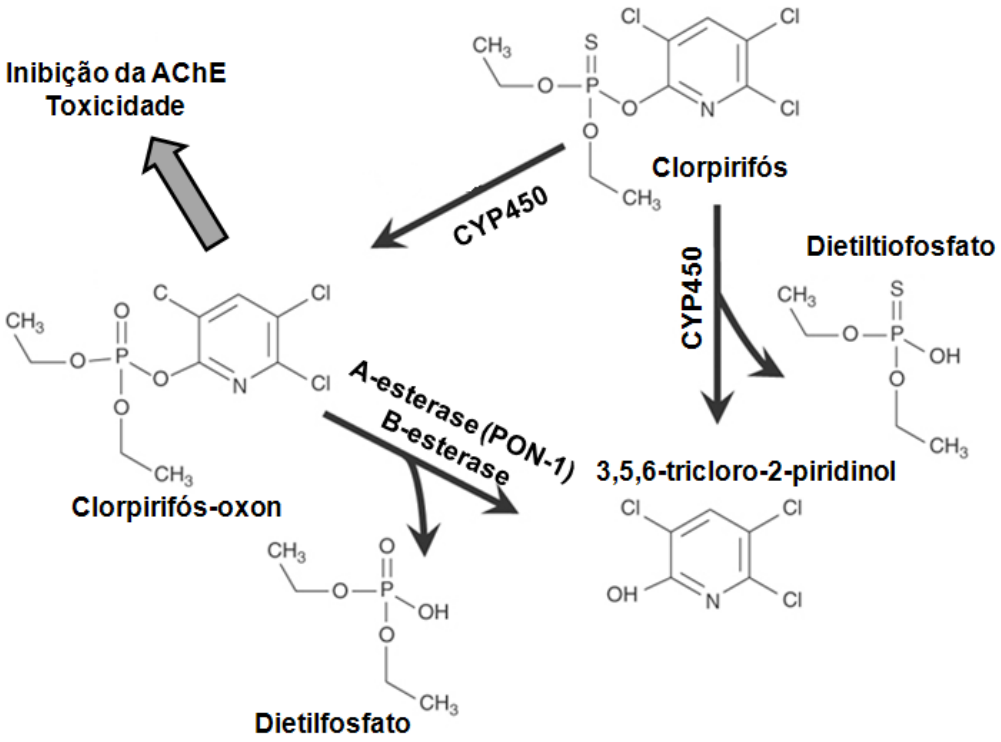

Figura 1 – Biotransformação do clorpirifós (CPF).
Fonte: Adaptado de Barry *et al*., 2009.

O AC, O,S-dimetil-N-acetil fosforamidotioato (Figura 2), é utilizado na agricultura para o controle de uma ampla variedade de insetos. Encontra-se como ingrediente ativo de mais de 100 diferentes formulações disponíveis no mercado. Pertence à classe toxicológica III (moderadamente tóxico) e apresenta uma DL_{50} de 866 mg/kg quando administrado pela via oral em ratos (Tomlin, 1995). A toxicidade do AC é atribuída à sua biotransformação à metamidofós, o qual age como um inibidor da AChE muito mais potente (Mahajna *et al*., 1997).

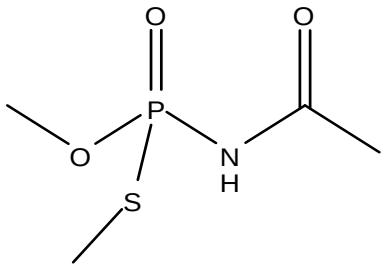

Figura 2 – Estrutura química do acefato (AC).

Alguns estudos têm demonstrado que o AC causa efeitos tóxicos quando administrado em animais experimentais, como genotoxicidade (Rahman *et al*., 2002), neurotoxicidade (Chen *et al*., 2003) e estresse oxidativo (Datta *et al*., 2010). Com relação ao metabolismo de carboidratos, Joshi e Rajini (2009) demonstraram que o AC causa hiperglicemia temporária após a sua administração em ratos. Além disso, esses autores demonstraram que os mecanismos envolvidos na hiperglicemia induzida pelo AC são a ativação do eixo HPA e da gliconeogênese, evidenciado pelo aumento dos níveis de corticosterona no plasma e da atividade das enzimas TAT e G6Pase no fígado dos ratos.

Com base na toxicidade causada pelos OFs, especialmente pelo CPF e pelo AC, torna-se necessária a busca de novas alternativas para reduzir os efeitos tóxicos destes. Dessa forma, destacam-se os compostos orgânicos de selênio, que nos últimos anos têm despertado grande interesse, não somente devido a sua importância como intermediários em síntese orgânica, mas também por apresentarem propriedades farmacológicas em diversos modelos experimentais (Nogueira *et al*., 2004; Nogueira e Rocha, 2010). Dentre os compostos de selênio que possuem ação farmacológica destaca-se o disseleneto de difenila [$(PhSe)_2$] (Figura 3).

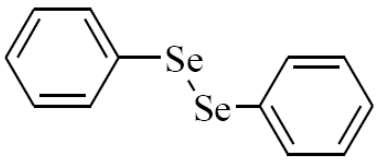

Figura 3 – Estrutura química do disseleneto de difenila [$(PhSe)_2$].

Estudos em animais de laboratório têm demonstrado que o $(PhSe)_2$ apresenta importantes propriedades, dentre as quais neuroprotetora (Ghisleine *et al*., 2003), anti-hiperglicêmica (Barbosa *et al*., 2006), hepatoprotetora (Borges *et al*., 2008), anti-hiperlipidêmica (da Rocha *et al*., 2009), antioxidante (Prigol *et al*., 2009a), entre outras. Recentemente foi demonstrado que o $(PhSe)_2$ inibe enzimas do citocromo P450, indicando que esse composto pode interferir no metabolismo de alguns agentes que dependem de metabolização para exercer sua toxicidade (Prigol, 2010).

Com relação à atividade anti-hiperglicêmica, Barbosa *et al*., 2006 demonstraram que o $(PhSe)_2$ (1 mg/kg) administrado pela via subcutânia durante 30 e 45 dias reduziu a hiperglicemia induzida por estreptozotocina em ratos. Barbosa *et al*., 2008 mostraram que a pré-administração e a pós-administração de $(PhSe)_2$ (10 mg/kg) pela via subcutânea durante 6 dias reduziu a hiperglicemia induzida por aloxano em ratos. Considerando-se a atividade anti-hiperlipidêmica, da Rocha *et al*., 2009 mostraram que a pré-administração de $(PhSe)_2$ (10 mg/kg) pela via intragástrica reduziu os níveis de CT, colesterol não-HDL e TG e aumentou os níveis de HDL no plasma de camundongos com hiperlipidemia induzida por triton.

Embora o $(PhSe)_2$ apresente consideráveis propriedades farmacológicas, trabalhos também têm evidenciado a ocorrência de efeitos tóxicos. A inibição da atividade das enzimas δ-aminolevulinato desidratase (δ-ALA-D) (Nogueira *et al*., 2003a) e Na^+, K^+, ATPase (Borges *et al*., 2005) foi observada e o potencial pró-oxidante do $(PhSe)_2$ parece estar envolvido nestes efeitos. Efeitos neurotóxicos do $(PhSe)_2$ também foram relatados (Nogueira *et al*., 2003b), incluindo a indução de convulsões (Prigol *et al*., 2008). Entretanto, tais efeitos neurotóxicos são observados apenas quando o $(PhSe)_2$ é administrado em doses altas (500 mg/kg) (Prigol *et al*., 2009b).

Com relação à farmacocinética do $(PhSe)_2$, Prigol *et al*. (2009b) demonstraram que o pico plasmático de $(PhSe)_2$ após a administração oral (500 mg/kg) foi em 30 min e a concentração plasmática máxima do composto foi de 13,13 e 10,11 μg/ml para ratos e camundongos, respectivamente. Foi demonstrado também que o $(PhSe)_2$ na dose de 500 mg/kg atinge concentrações no plasma, no fígado e no cérebro de 3,67, 5,07 e 1,15 μg/ml, respectivamente, após a administração pela via oral em filhotes de ratos (Prigol *et al*., 2009b).

Considerando que os OFs causam diversos efeitos tóxicos, torna-se de fundamental importância avaliar os efeitos farmacológicos do $(PhSe)_2$ como uma alternativa para reduzir a toxicidade dos OFs, bem como, avaliar os efeitos hiperglicêmico e hiperlipidêmico do CPF, que ainda não foram descritos.

2 OBJETIVOS

2.1 Objetivo Geral

Tendo em vista que a exposição à OFs induz diversos efeitos tóxicos, o objetivo do presente trabalho foi avaliar os efeitos farmacológicos do $(PhSe)_2$ em modelos de toxicidade induzida por CPF e AC em ratos, bem como, os efeitos hiperglicêmico e hiperlipidêmico do CPF.

2.2 Objetivos Específicos

I. Determinar os efeitos do $(PhSe)_2$ no estresse oxidativo hepático e nas alterações hematológicas induzidas pelo CPF após uma exposição aguda;
II. Avaliar os efeitos do $(PhSe)_2$ nos distúrbios metabólicos induzidos pelo AC;
III. Avaliar o efeito hiperglicêmico e hiperlipidêmico do CPF após uma exposição aguda;
IV. Investigar os mecanismos envolvidos na hiperglicemia induzida pelo CPF.

3 ARTIGOS CIENTÍFICOS

Os resultados que fazem parte desta tese estão apresentados sob a forma de artigos científicos. Os itens Resumo, Introdução, Materiais e Métodos, Resultados, Discussão dos Resultados e Referências encontram-se nos próprios artigos. Os artigos estão dispostos da mesma forma que foram publicados nas respectivas revistas científicas.

3.1 Artigo 1

O disseleneto de difenila atenua a toxicidade hepática e hematológica induzida pela exposição aguda ao clorpirifós em ratos

DIPHENYL DISELENIDE ATTENUATES HEPATIC AND HEMATOLOGIC TOXICITY INDUCED BY CHLORPYRIFOS ACUTE EXPOSURE IN RATS

Carmine Inês Acker, Ana Cristina Guerra Souza, Maurício Portella dos Santos, Cinthia Melazzo Mazzanti, Cristina Wayne Nogueira

Environmental Science and Pollution Research 19 (2012) 3481-3490

Environ Sci Pollut Res (2012) 19:3481–3490
DOI 10.1007/s11356-012-0882-4

RESEARCH ARTICLE

Diphenyl diselenide attenuates hepatic and hematologic toxicity induced by chlorpyrifos acute exposure in rats

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Received: 21 July 2011 / Accepted: 18 March 2012 / Published online: 5 April 2012

Abstract
Purpose In this study, we investigated the effect of diphenyl diselenide [$(PhSe)_2$] on chlorpyrifos (CPF)-induced hepatic and hematologic toxicity in rats.
Methods Rats were pre-treated with $(PhSe)_2$ (5 mg/kg) via the oral route (oral gavage) once a day for 7 days. On the eighth and ninth days, rats were treated with $(PhSe)_2$ (5 mg/kg) 30 min prior to CPF (50 mg/kg, by subcutaneous route). The aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase activities were determined in plasma of rats. Lipid peroxidation, protein carbonyl, and non-protein thiol levels as well as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and gluthatione S-transferase activities were determined in livers of rats. Hematological parameters were also determined.
Results The results showed that CPF caused hepatic oxidative damage, as demonstrated by an increase in lipid peroxidation and protein carbonyl levels which was associated with a decrease in antioxidant defenses. CPF exposure caused a reduction in the leukocyte, indicating hematologic toxicity. $(PhSe)_2$ was effective in attenuating these toxic effects caused by CPF exposure in rats.
Conclusions The results indicated that $(PhSe)_2$ was effective in protecting the hepatic and hematologic toxicity induced by acute CPF exposure in rats.

Keywords Pesticides · Chlorpyrifos · Selenium · Organoselenium · Liver damage · Hematologic

Responsible editor: Markus Hecker

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1 Introduction

Chlorpyrifos (CPF) is a broad-spectrum organophosphate (OP) insecticide; is extensively being used to control agricultural pests and disease vectors; and is preferred to chlorinated hydrocarbons for field applications because of its quick action, relatively shorter half-life and poor-accumulation in the food web (Kwong 2002; Miglioranza et al. 2002). It is metabolically activated in liver to its corresponding oxygen analog, CPF-oxon, which is primarily responsible for the mammalian toxicity through inhibition of acetylcholinesterase in the peripheral and central nervous system (Kousba et al. 2004).

Although cholinesterase inhibition is the main mechanism implicated in OPs toxicity, other mechanisms were reported (Lukaszewicz-Hussain 2010). Many authors postulate that OPs in acute and chronic intoxication disturb the redox processes, changing the activity of antioxidant enzymes and causing enhancement of lipid peroxidation in many organs (Soltatinejad and Abdollahi 2009; Lukaszewicz-Hussain 2010). In fact, CPF has been reported to have multiple effects on the target cells including generation of reactive oxygen species and induction of intracellular oxidative stress (Khan and Kour 2007). In this regard, previous studies have demonstrated the toxic actions of CPF to several systems and organs, including the liver, kidney, muscles, immune system, and hematologic system (Goel et al. 2006; Verma et al. 2007).

Liver is a major site for metabolism of exogenous chemicals (pesticides, drugs, and metals), resulting in the formation of metabolites which may be more or less toxic than the parent compound. It is also, apart from the gastrointestinal tract, the first major organ to be exposed to ingested toxins due to its portal blood supply and toxins may be, at least partially, removed from the circulation during the first pass, providing protection to other organs while increasing the likelihood of hepatic injury (Ncibi et al. 2008). Considering that the hepatic damage caused by xenobiotics, including OPs, usually involves the development of oxidative stress, antioxidants have been used in the attempt to protect the liver against the injury induced by these compounds (Khan and Kour 2007; Aly et al. 2010).

In this context, it is important to highlight the compound diphenyl diselenide [$(PhSe)_2$], a simple organoselenium compound, that has been reported due to its pharmacological properties (Nogueira and Rocha 2010) which include the following: anti-hyperglycemiant (Barbosa et al. 2006), anti-hyperlipidaemic (da Rocha et al. 2011), antioxidant (Prigol et al. 2009a), renoprotector (Brandão et al. 2009a), and antidepressant (Acker et al. 2009) in rodents. Moreover, studies from our research group have demonstrated that $(PhSe)_2$ protects against hepatic injury and hematologic alterations induced by toxic agents (Borges et al. 2008; Brandão et al. 2008).

In view of the above considerations, the aim of the present study was to investigate the effect of $(PhSe)_2$ on hepatic and hematologic toxicity induced by CPF acute exposure in rats.

2 Materials and methods

2.1 Chemicals

CPF (La Forja S.A.) was obtained from commercial grade. The purity of CPF commercial pesticide (47.2 %) was determined by gas chromatography (GC) with flame ionization detection according to CIPAC Handbook, volume H (1998). $(PhSe)_2$ was prepared in our laboratory according to Paulmier (1986) and the chemical purity (99.9 %) was determined by GC/MS. Analysis of ^{1}H and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. $(PhSe)_2$ and CPF were dissolved in canola oil and saline, respectively. All other chemicals were obtained from standard commercial suppliers.

2.2 Animals

Male adult Wistar rats, weighing 200–300 g, were obtained from a local breeding colony. Animals were kept in a separate animal room, on a 12-h light/12-dark cycle with lights on at 7:00 a.m., in an air-conditioned room (22±2 °C). Commercial diet (Guabi, RS, Brasil) and tap water were supplied ad libitum. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources and with the approval of the Animal Use Committee (23081.017070/2011-19), Federal University of Santa Maria, Brazil.

2.3 Experimental procedure

Rats were divided into four groups of seven to nine animals each. The animals were pre-treated once a day for seven consecutive days with $(PhSe)_2$ (5 mg/kg; by oral gavage). On the eighth and ninth days, rats were treated with $(PhSe)_2$ (5 mg/kg) 30 min prior to the subcutaneous (s.c.) administration of CPF (50 mg/kg of active ingredient). Pre-treatment time of 30 min for $(PhSe)_2$ administration was based on a previous pharmacokinetic study with this compound (Prigol et al. 2009b). The dose of $(PhSe)_2$ which does not cause toxicity in rodents and presents pharmacological effects was chosen based on a previous study of our research group (da Rocha et al. 2011). The dose of CPF was based on a pilot study performed by our research group. Due to biological variation between animals, we performed a pilot study to determine the CPF dose and exposure period necessary to cause liver and hematologic toxicity under our experimental conditions. This dose represents approximately 1/3 of reported lethal dose (LD_{50}) (Eaton et al. 2008).

The protocol of rat treatment is given below:

- Group I: canola oil (1 ml/kg; p.o.) plus saline 0.9 % (1 ml/kg; s.c.);
- Group II: $(PhSe)_2$ (5 mg/kg; p.o.) plus saline 0.9 % (1 ml/kg; s.c.);
- Group III: canola oil (1 ml/kg; p.o.) plus CPF (50 mg/kg; s.c.);
- Group IV: $(PhSe)_2$ (5 mg/kg; p.o.) plus CPF (50 mg/kg; s.c.).

The animals were observed for signs of CPF toxicity (weight loss, salivation, tremors, and death). Twenty-four hours after the last CPF injection, all rats were anesthetized for blood collection by heart puncture (hemolyzed plasma was discharged). After this procedure, the rats were killed and the livers of animals were removed, dissected, and kept on ice until the time of assay. The liver samples were homogenized in 50 mM Tris–HCl (pH 7.4; 1:10*w*/*v*) and centrifuged at 2,400×*g* for 10 min, except for protein carbonyl content determination in which the homogenate was used without centrifugation. The low-speed supernatants (S_1) were separated and used for the other biochemical assays.

2.4 Hepatic and cellular markers of damage

Plasma activities of alanine and aspartate aminotransferases (ALT and AST, respectively) and lactate dehydrogenase (LDH) were assayed spectrophotometrically according to Reitman and Frankel (1957) using a commercial kit (Labtest, Diagnostica S.A., Minas Gerais, Brazil).

2.5 Hepatic lipid peroxidation levels determination

A 200 μl aliquot of S_1 was added to the reaction mixture containing 500 μl of 0.8 % thiobarbituric acid, 200 μl of 8.1 % sodium dodecyl sulfate, and 500 μl of acetic acid (pH 3.4), and was incubated at 95 °C for 2 h. Thiobarbituric-acid-reactive species were determined as described by Ohkawa et al. (1979). Malondialdehyde (MDA), formed as an end product of lipid peroxidation and served as an index of the intensity of lipid peroxidation. MDA reacts with thiobarbituric acid to generate a colored product that can be measured optically at 532 nm. Lipid peroxidation levels were expressed as nanomoles of MDA per milligram of protein.

2.6 Hepatic protein carbonyl levels determination

Liver homogenates (1/10, *w*/*v*) were prepared in 50 mM Tris–HCl buffer, pH 7.4. The protein carbonyl determination was carried out as described by Reznick and Packer (1994). Aliquots of 1,000 μl of the diluted homogenates were incubated in three tubes. In two tubes, it was added 200 μl of 10 mM 2,4-dinitrophenylhydrazine in 2.0 M HCl. The other tube contains only 200 μl of 2.0 M HCl solution (blank). Tubes were incubated for 60 min at room temperature in dark and were shaken with a Vortex mixer every 15 min. After that, 0.5 ml of denaturizing buffer (sodium phosphate buffer, pH 6.8, containing 3 % sodium dodecyl sulfate (SDS)), 1.5 ml of ethanol, and 1.5 ml of hexane were added. The mixture was shaken with a vortex mixer for 40 s and centrifuged at 2,400×*g* for 15 min. The pellet obtained was separated and washed two times with 1 ml of ethanol: ethyl acetate (1:1, *v*/*v*). The pellet was dissolved in 1 ml of denaturizing buffer solution with mixing. Absorbance was measured at 370 nm. Protein carbonyl levels were expressed as nanomoles of carbonyl per milligram of protein.

2.7 Hepatic non-protein thiol (NPSH) levels determination

NPSH levels were determined by the method of Ellman (1959). An aliquot of S_1 was mixed (1:1) with 10 % trichloroacetic acid (TCA) and centrifuged at 4,000×*g* 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 1 M potassium phosphate buffer, pH 7.4, and 10 mM 5,5-dithiobis(2-nitrobenzoic acid) (DTNB). The color reaction was measured at 412 nm. NPSH levels were expressed as micromoles of NPSH per gram of tissue.

2.8 Hepatic catalase (CAT) activity assay

CAT activity was spectrophotometrically determined by the method of Aebi (1984), which involves monitoring the disappearance of H_2O_2 in the homogenate presence at 240 nm. Enzymatic reaction was initiated by adding an aliquot of S_1 and the substrate (H_2O_2) to a concentration of 0.3 mM in a medium containing 50 mM potassium phosphate buffer, pH 7.0. The enzymatic activity was expressed as Units (U) per milligram of protein (1 U decomposes 1 μmol of H_2O_2 per minute at pH 7.0 and 25 °C).

2.9 Hepatic superoxide dismutase (SOD) activity assay

SOD activity in S_1 was spectrophotometrically determined as described by 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 S_1 was diluted 1:10 (*v*/*v*) for determination of SOD activity in the test day. Aliquots of supernatant were added in a 50 mM Na_2CO_3 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) per milligram of protein.

2.10 Hepatic glutathione peroxidase (GPx) activity assay

GPx activity was spectrophotometrically determined by the method of Wendel (1981), through the reduced glutathione (GSH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH) or glutathione reductase (GR) system, by the dismutation of H_2O_2 at 340 nm. S_1 was added in GSH/NADPH/GR 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 oxidized glutathione (GSSG) from GSH. GSSG is regenerated back to GSH by GR present in the assay media at the expenses of NADPH. The enzymatic activity was expressed as nanomoles of NADPH per minute per milligram of protein.

2.11 Hepatic glutathione reductase (GR) activity assay

GR activity was determined as described by Carlberg and Mannervik (1985). In this assay, GSSG is reduced by GR at

the expense of NADPH consumption, which is followed at 340 nm. GR activity is proportional to NADPH decay. The enzymatic activity was expressed as nanomoles of NADPH per minute per milligram of protein.

2.12 Hepatic glutathione S-transferase (GST) activity assay

GST activity was assayed spectrophotometrically at 340 nm by the method of Habig et al. (1974). The reaction mixture contained an aliquot of S_1, 0.1 M potassium phosphate buffer pH 7.4, 100 mM GSH and 100 mM 1-chloro-2,4-dinitrobenzene (CDNB), which was used as substrate. The enzymatic activity was expressed as nanomoles of CDNB conjugated per minute per milligram of protein.

2.13 Protein quantification

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

2.14 Hematologic parameters determination

Erythrocyte counts, hematocrit, total leukocytes and differential leukocyte were performed. The white blood cells and erythrocyte counts were carried out in an automatic counter Mindray BC 2800 Vet. Determination of hematocrit was obtained from microhematocrit centrifuge in 19,720×*g* rotation, and the differential count was performed on blood smears, stained with Panoptic method, using light microscopy.

2.15 Statistical analysis

Statistical analysis was performed using a two-way analysis of variance (ANOVA), followed by the Duncan's multiple range test. Main effects are presented only when the higher second-order interaction was non-significant. Data were expressed as the mean(s)±S.E.M. of seven to nine animals. A value of $p<0.05$ was considered to be significant.

3 Results

3.1 Signs of toxicity

No mortality occurred during the experimental period. Signs of cholinergic toxicity (tremors, weakness, and diarrhea) were observed in animals exposed to CPF, but $(PhSe)_2$ was ineffective against these signs. Concerning the body weight of rats, $(PhSe)_2$ partially protected against the weight loss caused by CPF (Table 1).

Table 1 Effects of $(PhSe)_2$ on body weight of rats exposed to CPF

Group	Final body weight (g)	Body weight gain or loss (%)
Control	287.57±7.07	8.42±2.45
$(PhSe)_2$	294.85±4.71	9.00±2.09
CPF	234.42±6.75*	−14.00±1.68*
CPF+$(PhSe)_2$	257.22±7.59*, **	−1.22±1.85*, **

Data are reported as mean±S.E.M. of seven to nine animals per group

*$p<0.05$ as compared to the control group, **$p<0.05$ as compared to the CPF group (two-way ANOVA/Duncan's multiple range test)

3.2 Hepatic and cellular markers of damage

ALT activity data demonstrated a significant $(PhSe)_2$×CPF interaction ($F_{1,27}=23.19$, $p<0.0001$). Post hoc comparisons revealed that CPF increased ALT activity of rats when compared to that of the control group. $(PhSe)_2$ reduced ALT activity increased by CPF (Table 2).

AST activity data showed a significant $(PhSe)_2$×CPF interaction ($F_{1,27}=43.84$, $p<0.00001$). Post hoc comparisons revealed that CPF increased AST activity when compared to that of the control group. $(PhSe)_2$ partially attenuated AST activity increased by CPF (Table 2).

LDH activity results yielded a significant $(PhSe)_2$×CPF interaction ($F_{1,27}=7.69$, $p<0.01$). Post hoc comparisons demonstrated that CPF increased LDH activity when compared to that of the control group. $(PhSe)_2$ reduced LDH activity increased by CPF (Table 2).

$(PhSe)_2$ at a dose of 5 mg/kg, for nine consecutive days, did not alter plasma ALT, AST, and LDH activities (Table 2).

3.3 Hepatic lipid peroxidation levels

The two-way ANOVA data of lipid peroxidation levels revealed a significant $(PhSe)_2$×CPF interaction ($F_{1,27}=$

Table 2 Effects of $(PhSe)_2$ on ALT, AST and LDH activities in plasma of rats exposed to CPF

Group	ALT	AST	LDH
Control	61.67±3.32	96.28±5.89	410.68±27.17
$(PhSe)_2$	65.71±3.93	109.00±5.64	488.51±46.27
CPF	111.50±8.87*	427.62±32.10*	666.51±74.71*
CPF+$(PhSe)_2$	62.11±3.74**	197.55±11.23*, **	451.19±44.77**

Data are reported as mean±S.E.M. of seven to nine animals per group and expressed as Units per liter

*$p<0.05$ as compared to the control group, **$p<0.05$ as compared to the CPF group (two-way ANOVA/Duncan's multiple range test)

8.10, $p<0.01$). Post hoc comparisons showed that CPF exposure increased basal lipid peroxidation levels. $(PhSe)_2$ decreased lipid peroxidation to basal levels when compared to those of the CPF group (Fig. 1a).

The lipid peroxidation levels in $(PhSe)_2$ and all other experimental groups were similar (Fig. 1a).

3.4 Hepatic protein carbonyl levels

The two-way ANOVA of protein carbonyl data demonstrated a significant $(PhSe)_2 \times CPF$ interaction ($F_{1,27}=17.60$, $p<0.001$). Post hoc comparisons showed that CPF increased protein carbonyl levels when compared to those of the control group. $(PhSe)_2$ treatment protected against the increase of protein carbonyl levels caused by CPF (Fig. 1b).

$(PhSe)_2$ at a dose of 5 mg/kg did not alter protein carbonyl levels in livers of rats (Fig. 1b).

3.5 Hepatic NPSH levels

The two-way ANOVA of NPSH levels data revealed a significant $(PhSe)_2 \times CPF$ interaction ($F_{1,27}=15.85$, $p<0.001$). Post hoc comparisons showed that CPF reduced NPSH levels of rats when compared to those of the control group. $(PhSe)_2$ treatment protected against the decrease of NPSH levels caused by CPF (Fig. 2a).

$(PhSe)_2$ at a dose of 5 mg/kg, for nine consecutive days, increased NPSH levels in livers of rats (Fig. 2a).

3.6 Hepatic CAT activity

The two-way ANOVA of CAT activity yielded a significant $(PhSe)_2 \times CPF$ interaction ($F_{1,27}=7.29$, $p<0.05$). Post hoc comparisons revealed that $(PhSe)_2$ treatment partially protected against CAT inhibition induced by CPF (Fig. 2b).

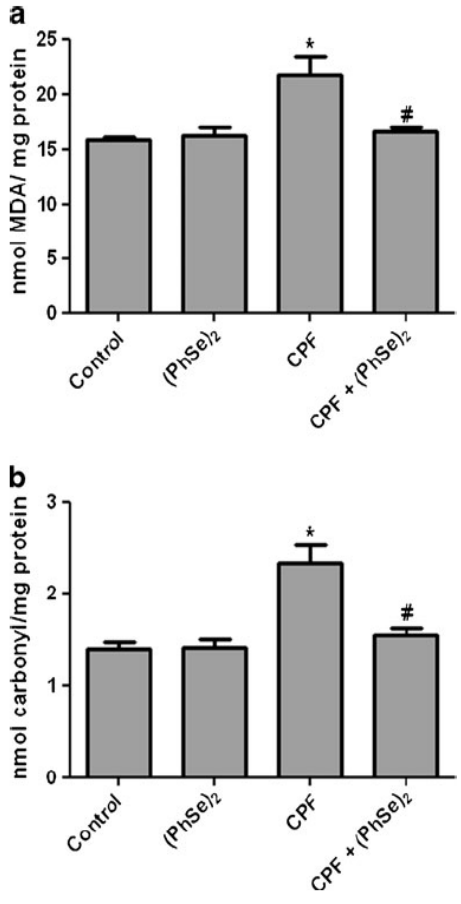

Fig. 1 Effect of $(PhSe)_2$ on lipid peroxidation (**a**) and protein carbonyl (**b**) levels in livers of rats exposed to CPF. Data are reported as the mean(s)±S.E.M. of seven to nine animals per group and expressed as nanomoles of MDA per milligram protein and nanomoles carbonyl per milligram protein, respectively. *$p<0.05$ as compared to the control group. #$p<0.05$ as compared to the CPF group (two-way ANOVA/ Duncan's multiple range test)

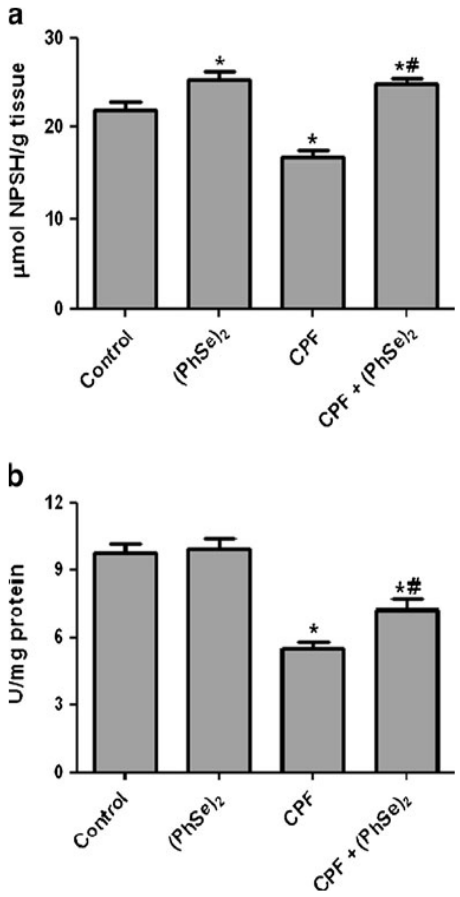

Fig. 2 Effect of $(PhSe)_2$ on NPSH levels (**a**) and CAT activity (**b**) in livers of rats exposed to CPF. Data are reported as the mean (s)±S.E.M. of seven to nine animals per group and expressed as micromoles of NPSH per gram tissue and units per milligram of protein, respectively. *$p<0.05$ as compared to the control group. #$p<0.05$ as compared to the CPF group (two-way ANOVA/Duncan's multiple range test)

$(PhSe)_2$ at a dose of 5 mg/kg did not alter CAT activity in livers of rats (Fig. 2b).

3.7 Hepatic SOD activity

The two-way ANOVA data of SOD activity yielded a significant $(PhSe)_2 \times CPF$ interaction ($F_{1,27}=12.37$, $p<0.01$). Post hoc comparisons revealed that CPF inhibited SOD activity when compared to the control group. $(PhSe)_2$ treatment protected against the inhibition of SOD activity resulting from CPF exposure (Fig. 3a).

The hepatic SOD activity was not altered by $(PhSe)_2$ treatment (Fig. 3a).

3.8 Hepatic GPx activity

The two-way ANOVA of GPx activity showed a significant $(PhSe)_2 \times CPF$ interaction ($F_{1,27}=8.40$, $p<0.01$). Post hoc comparisons demonstrated that CPF inhibited GPx activity when compared to that of the control group. $(PhSe)_2$ treatment protected against the inhibition of GPx activity caused by CPF (Fig. 3b).

GPx activity in $(PhSe)_2$ and all other experimental groups was similar (Fig. 3b).

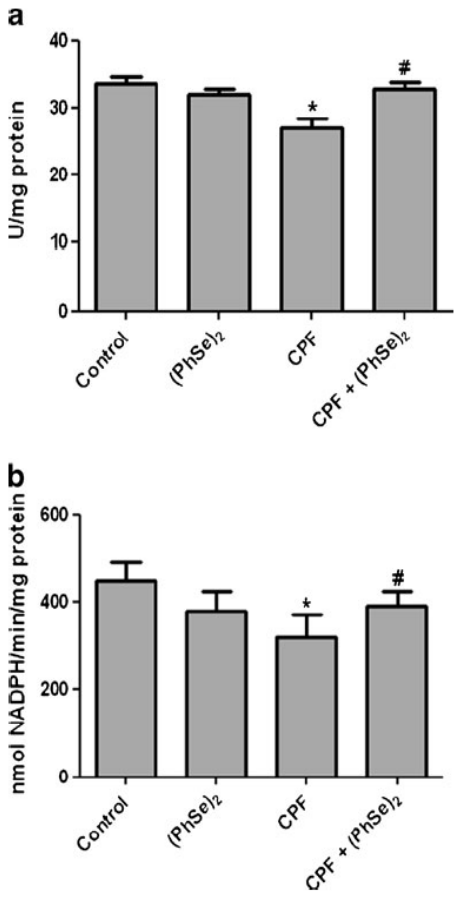

Fig. 3 Effect of $(PhSe)_2$ on SOD (**a**) and GPx (**b**) activities in livers of rats exposed to CPF. Data are reported as the mean (s)±S.E.M. of seven to nine animals per group and expressed as units per milligram protein and nanomoles of NADPH per minute per milligram of protein, respectively. *$p<0.05$ as compared to the control group. #$p<0.05$ as compared to the CPF group (two-way ANOVA/Duncan's multiple range test)

3.9 Hepatic GR activity

Two-way ANOVA of GR activity demonstrated that CPF exposure did not alter the enzyme activity in livers of rats (data not shown).

3.10 Hepatic GST activity

The two-way ANOVA of GST activity revealed a significant main effect of CPF ($F_{1,27}=9.28$, $p<0.01$) and $(PhSe)_2$ ($F_{1,27}=23.28$, $p<0.0001$) in livers of rats. Post hoc comparisons showed that $(PhSe)_2$ treatment was effective against the inhibition of GST activity caused by CPF (Fig. 4).

$(PhSe)_2$ treatment increased GST activity in livers of rats (Fig. 4).

3.11 Hematologic parameters

The two-way ANOVA of hemoglobin levels yielded a significant main effect of CPF ($F_{1,20}=47.20$, $p<0.0001$). Post hoc comparisons showed that CPF increased hemoglobin levels when compared to those of the control group. $(PhSe)_2$ treatment was not effective against the increase of hemoglobin levels caused by CPF (Table 3).

The two-way ANOVA of hematocrit levels showed a significant main effect of CPF ($F_{1,20}=34.73$, $p<0.00001$). Post hoc comparisons revealed that CPF increased hematocrit

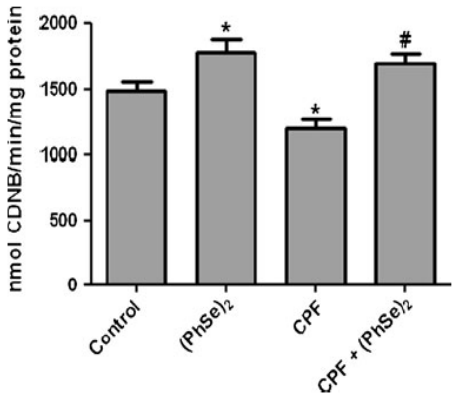

Fig. 4 Effect of $(PhSe)_2$ on GST activity in liver of rats exposed to CPF. Data are reported as the mean(s)±S.E.M. of seven to nine animals per group and expressed as nanomoles of CDNB per minute per milligram of protein. *$p<0.05$ as compared to the control group. #$p<0.05$ as compared to the CPF group (two-way ANOVA/Duncan's multiple range test)

levels when compared to those of the control group. $(PhSe)_2$ treatment was ineffective against the increase of hematocrit levels caused by CPF (Table 3).

The two-way ANOVA of leukocyte levels demonstrated a significant $(PhSe)_2 \times CPF$ interaction ($F_{1,20}=8.32$, $p<0.01$). Post hoc comparisons showed that CPF decreased leukocyte levels when compared to those of the control group. $(PhSe)_2$ treatment attenuated partially the decrease of leukocyte levels caused by CPF (Table 3).

The two-way ANOVA of lymphocyte levels yielded a significant main effect of CPF ($F_{1,20}=15.67$, $p<0.001$). Post hoc comparisons showed that treatment with $(PhSe)_2$ was ineffective against the decrease of lymphocyte levels caused by CPF (Table 3).

The two-way ANOVA of the levels of monocytes revealed a significant main effect of CPF ($F_{1,20}=9.86$, $p<0.01$). Post hoc comparisons showed that CPF increased monocyte levels when compared to those of the control group. $(PhSe)_2$ treatment attenuated the increase of monocyte levels caused by CPF (Table 3).

The two-way ANOVA of neuthrophil levels showed a significant main effect of CPF ($F_{1,20}=15.54$, $p<0.001$). Post hoc comparisons showed that CPF increased the levels of neuthrophils when compared to those of the control group. $(PhSe)_2$ treatment was not effective against the increase of neuthrophil levels caused by CPF (Table 3).

The two-way ANOVA of erythrocyte, platelet, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) values were similar in all experimental groups (Table 3).

$(PhSe)_2$ treatment at a dose of 5 mg/kg did not alter hematological parameters of rats (Table 3).

4 Discussion

In the current study, we reported the protective effect of $(PhSe)_2$ on hepatic and hematologic toxicity caused by CPF exposure in rats. CPF exposure caused a significant hepatotoxicity, as demonstrated by an increase in plasma AST, ALT, and LDH activities, hepatic and cellular markers of damage. Moreover, CPF exposure caused hepatic oxidative damage, clearly demonstrated by an increase in lipid peroxidation and protein carbonyl levels which was associated with a decrease in antioxidant defenses. In addition, CPF exposure reduced markedly the levels of leukocytes, indicating hematologic toxicity.

CPF is a widely used insecticide, which is primarily metabolized in the liver involving the intervention of specific cytochrome P450s through several pathways (Mutch and Williams 2007). Some studies have reported the hepatotoxicity induced by CPF exposure in rats (Mansour and Mossa 2010; Tripathi and Srivastav 2010). The results of the present study revealed that CPF exposure caused an increase in the activity of ALT, AST, and LDH in plasma of rats. The increase of ALT, AST, and LDH activities indicates liver damage with enhancement in the permeability of hepatocyte membranes leading to leakage of these enzymes into the blood stream (Gokcimen et al. 2007). Plasma enzymes, including AST, ALT, and LDH, are mainly used in the evaluation of hepatic damage (Sutcu et al. 2006). The results demonstrated in this study showed that $(PhSe)_2$ completely attenuated the increase in AST and LDH activities and partially attenuated the increase in AST activity, indicating its hepatoprotective activity. Accordingly, this organoselenium compound has been proven to protect against hepatotoxicity induced by other chemicals (Borges et al. 2008; Brandão et al. 2008). The partial attenuation of the increase in AST activity by $(PhSe)_2$ may be explained by the fact that AST is a mitochondrial enzyme, an organelle located more internally in the cell and therefore less accessible to $(PhSe)_2$.

In this study, CPF exposure caused hepatic oxidative damage, as demonstrated by the increase in lipid peroxidation and protein carbonyls levels. $(PhSe)_2$ normalized these parameters in the liver of exposed rats, suggesting that the antioxidant activity of $(PhSe)_2$ is involved in protecting against hepatic damage induced by CPF exposure in rats. In this sense, some studies have proven that OPs induce

Table 3 Effects of $(PhSe)_2$ on hematological parameters of rats exposed to CPF

Parameters	Control	$(PhSe)_2$	CPF	CPF+$(PhSe)_2$
Erythrocytes (10^6/μl)	6.62±0.39	7.33±0.19	7.42±0.37	7.66±0.22
Hemoglobin (g/dl)	13.64±0.22	14.06±0.48	16.30±0.31*	16.07±0.27*
Hematocrit (%)	42.16±1.13	43.66 ± 1.28	49.16±0.83*	48.83±0.79*
MCV (fl)	64.41±3.07	59.51±0.24	66.92±3.06	64.69±2.63
MCHC (%)	32.40±0.55	32.22±0.62	33.14±0.10	32.92±0.38
Platelets (10^3/μl)	1.16±0.04	1.26±0.04	1.15±0.03	1.26±0.11
Leukocytes (10^3/μl)	14.43±1.35	11.76±1.08	4.76±0.68*	7.96±0.62*, **
Lymphocytes (%)	74.66±2.81	67.00±8.18	51.83±5.16*	50.50±6.10*
Monocytes (%)	3.00±1.02	3.33±0.91	10.16±2.37*	5.33±0.98**
Neutrophils (%)	32.66±12.83	33.00±5.58	57.33±16.56*	56.33±20.48*

Data are reported as mean± S.E.M. of six animals per group
*$p<0.05$ as compared to the control group, **$p<0.05$ as compared to the CPF group (two-way ANOVA/Duncan's multiple range test)

oxidative stress by generation of reactive species that can cause lipid peroxidation, alterations in membrane fluidity, structural modification of proteins, DNA damage, and apoptosis (Verma et al. 2007; Soltatinejad and Abdollahi 2009; Lukaszewicz-Hussain 2010). Regarding CPF, hepatic oxidative stress associated to an increase in lipid peroxidation has been reported after CPF exposure (Khan and Kour 2007; Aly et al. 2010).

The biological systems have several mechanisms to counteract the damage caused by reactive species. Antioxidant enzymes such as CAT, GPx, SOD, GR, and non-enzymatic antioxidants such as NPSH (mainly GSH) are the most important antioxidant defenses in biological systems (Halliwell 2011). Their concentrations are altered rapidly in the body during xenobiotic insults, resulting in their increase or decrease in the body tissues. In this study, the reduced levels of NPSH in the livers of rats intoxicated with CPF could be associated with the elevation of lipid peroxidation and protein carbonyl levels. $(PhSe)_2$ restored NPSH levels in liver of intoxicated rats reinforcing the protective role of this organoselenium compound against the oxidative damage induced by CPF. Furthermore, $(PhSe)_2$ caused an increase in NPSH levels, indicating an induction of NPSH-related antioxidant capacity by this compound. Accordingly, our research group has demonstrated similar effect of $(PhSe)_2$ in other experimental protocols (Barbosa et al. 2006; Luchese et al. 2009).

SOD plays an important role in the dismutation of superoxide radicals to form hydrogen peroxide and molecular oxygen, and acts as the first line of defense. CAT in turn converts the peroxide to molecular oxygen and water (Halliwell 2011). In the present study, SOD and CAT activities were inhibited in the livers of CPF-exposed rats. The presence of superoxide radicals has been shown to directly inhibit the activity of CAT while singlet oxygen and peroxyl radicals inhibit SOD and CAT activities (Khan et al. 2005). The excessive reactive species production induced by CPF exposure may explain the inhibition of CAT and SOD activities. Some authors have reported that CPF exposure causes the increase of antioxidant enzymes activities (Aly et al. 2010; Uzun et al. 2010). However, other studies provided evidence for the inhibition of these enzymes after CPF exposure (Khan and Kour 2007; Verma et al. 2007). In accordance with these authors in the present study, we demonstrated the inhibition of antioxidant enzymes in livers of rats. It is important to consider that the above-mentioned studies used different regimens (i.e., different CPF doses and treatment times), which could help to explain the controversial results. Therefore, the effect of CPF on antioxidant enzymes is variable and dependent on the protocol of exposure. On the other hand, treatment with $(PhSe)_2$ protected against the inhibition of SOD and CAT activities, suggesting that the hepatoprotective effect of this compound may involve its antioxidant action.

GPx is an antioxidant enzyme that has an important role in catalyzing the reaction of H_2O_2 and lipid hydroperoxides with GSH to form their reduced analogs and GSSG (Arthur 2000). GST is a detoxifying enzyme that catalyzes the conjugation of a variety of electrophilic substrates to the thiol group of GSH, producing less toxic forms (Hayes et al. 2005). In addition, GST can interrupt lipid peroxidation chain reactions through the detoxification of lipid hydroperoxides (Sharma et al. 2004). In the current study, we found an inhibition of GPx and GST activities in the livers of CPF-exposed rats. Since GSH is essential for the activity of these enzymes, the inhibition of GPx and GST activities can be attributed to the decrease in NPSH levels demonstrated in this study. $(PhSe)_2$ protected against the effects of CPF in the GSH system, i.e., increased the activity of GPx and GST as well as restored NPSH levels, reinforcing the idea that the antioxidant property is involved in the hepatoprotective action of this organoselenium compound. Moreover, $(PhSe)_2$ increased per se the GST activity and NPSH levels in the livers of rats, suggesting a plausible mechanism by which $(PhSe)_2$ acts as a protective agent in the livers of rats. Furthermore, it has been reported that the pharmacological activity of $(PhSe)_2$ mainly involves its interaction with thiol groups with a generation of intermediate selenol groups. These selenol–selenolate groups can decompose H_2O_2 and lipid peroxides formed during the propagation phase of lipid peroxidation (Nogueira and Rocha 2010).

Evidence has been found to suggest that OPs cause hematologic toxicity, such as alterations in erythrocyte and leukocyte counts (Çetin et al. 2010). The main hematologic alteration demonstrated in the current study was the reduction in total leukocyte and lymphocyte counts and the increase in neutrophil and monocyte percentages in CPF exposed rats. These results are in accordance with other studies that demonstrated hematologic alterations after CPF exposure (Goel et al. 2006; Ambali et al. 2007). The decreased levels of total leukocytes, a marker of cellular defense, may be due to a decreased rate of production of leukocytes or due to their decreased release into the blood stream. Since neutrophils and monocytes are the first and second line of defense against infectious agents and tissue injury (Kobayashi et al. 2003), one can suggest that the increase in neutrophil and monocyte levels indicates a cellular damage induced by CPF. Regarding the effect of $(PhSe)_2$ against these alterations, $(PhSe)_2$ attenuated the decrease of leukocyte levels and the increase of monocyte levels, demonstrating its immunomodulatory properties. Accordingly, $(PhSe)_2$ has been proven to protect against hematologic toxicity induced by other agents (Brandão et al. 2008, 2009b). It is difficult to exactly explain why hemoglobin and hematocrit values were increased in CPF-exposed rats; however, this discrete alteration may be a possible defense mechanism against CPF toxicity.

5 Conclusion

This study demonstrated that $(PhSe)_2$ was effective against hepatic oxidative damage and hematologic toxicity induced by CPF exposure in rats. The antioxidant role of $(PhSe)_2$ was proven to be involved in its hepatoprotective effect in rats exposed to CPF. These findings are of great importance since the crescent use of organophosphate insecticides worldwide has been the cause of many severe human poisoning cases and new therapies are needed to prevent these poisonings.

Acknowledgments The authors thank to Rio Grande do Sul State Research Foundation (FAPERGS), Brazilian Research Council (CNPq), FAPERGS/CNPq (PRONEX) research grant no. 10/0005-1 and FAPERGS 10/07116.

References

Acker CI, Luchese C, Prigol M, Nogueira CW (2009) Antidepressant-like effect of diphenyl diselenide on rats exposed to malathion: involvement of Na^+K^+ATPase activity. Neurosci Lett 455:168–172

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Aly N, El-Gendy K, Mahmoud F, El-Sebae AK (2010) Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. Pestic Biochem Physiol 97:7–12

Ambali S, Akanbi D, Igbokwe N, Shittu M, Kawu M, Ayo J (2007) Evaluation of subchronic chlorpyrifos poisoning on hematological and serum biochemical changes in mice and protective effect of vitamin C. J Toxicol Sci 32:111–120

Arthur JR (2000) The glutathione peroxidases. Cell Mol Life Sci 57:1825–1835

Barbosa NBV, Rocha JBT, Wondracek DC, Perottoni J, Zeni G, Nogueira CW (2006) Diphenyl diselenide reduces temporarily hyperglycemia: possible relationship with oxidative stress. Chem Biol Interact 163:230–238

Borges LP, Brandao R, Godoi B, Nogueira CW, Zeni G (2008) Oral administration of diphenyl diselenide protects against cadmium-induced liver damage in rats. Chem Biol Interact 171:15–25

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

Brandão R, Borges LP, de Oliveira R, Rocha JBT, Nogueira CW (2008) Diphenyl diselenide protects against hematological and immunological alterations induced by mercury in mice. J Biochem Mol Toxicol 22:311–319

Brandão R, Acker CI, Leite MR, Barbosa NBV, Nogueira CW (2009a) Diphenyl diselenide protects against glycerol-induced renal damage in rats. J Appl Toxicol 29:612–618

Brandão R, Borges LP, Nogueira CW (2009b) 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 47:1771–1778

Carlberg I, Mannervik B (1985) Glutathione reductase. Methods Enzymol 113:484–489

Çetin E, Kanbur M, Silici S, Eraslan G (2010) Propetamphos-induced changes in haematological and biochemical parameters of female rats: protective role of própolis. Food Chem Toxicol 48:1806–1810

CIPAC Handbook, Volume H (1998) Analysis of technical and formulated pesticides. In: W. Dobrat and A. Martijn (eds). Black Bear Press, King's Hedges Road, Cambridge, UK, 359 pp

Da Rocha JT, Pinton S, Mazzanti A, Mazzanti CM, Beckemann DV, Nogueira CW, Zeni G (2011) Effects of diphenyl diselenide on lipid profile and hepatic oxidative stress parameters in ovariectomized female rats. J Pharm Pharmacol 63:663–669

Eaton DL, Daroff RB, Autrup H, Bridges J, Buffler P, Costa LG, Coyle J, Mckhann G, Mobley WC, Nadel L, Neubert D, Schulte-Hermann R, Spencer PS (2008) Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit Rev Toxicol 38:1–125

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem 82:70–77

Goel A, Dani V, Dhawan DK (2006) Role of zinc in mitigating the toxic effects of chlorpyrifos on hematological alterations and electron microscopic observations in rat blood. Biometals 19:483–492

Gokcimen A, Gulle K, Demirin H, Bayram D, Kocak A, Altuntas I (2007) Effects of diazinon at different doses on rat liver and pancreas tissues. Pest Biochem Physiol 87:103–108

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione S-transferases, the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Halliwell B (2011) Free radicals and antioxidants—quo vadis? Trends Pharmacol Sci 32:125–130

Hayes JD, Flanagan JU, Jowsey IR (2005) Glutathione transferases. Annu Rev Pharmacol Toxicol 45:51–88

Khan SM, Sobti RC, Kataria L (2005) Pesticide-induced alteration in mice hepatooxidative status and protective effects of black tea extract. Clin Chim Acta 358:131–138

Khan SM, Kour G (2007) Subacute oral toxicity of chlorpyriphos and protective effect of green tea extract. Pest Biochem Phisiol 89:118–123

Kobayashi SD, Voyich JM, DeLeo FR (2003) Regulation of the neutrophil-mediate inflammatory response to infection. Microbes Infect 5:1337–1344

Kousba AA, Sultatos LG, Poet TS, Timchalk C (2004) Comparison of chlorpyrifos-oxon and paraoxon acetylcholinesterase inhibition dynamics: potential role of a peripheral binding site. Toxicol Sci 80:239–248

Kwong TC (2002) Organophosphate pesticides: biochemistry and clinical toxicology. Ther Drug Monit 24:144–149

Luchese C, Stangherlin EC, Gay BM, Nogueira CW (2009) Antioxidant effect of diphenyl diselenide on oxidative damage induced by smoke in rats: involvement of glutathione. Ecotoxicol Environ Saf 72:248–254

Lukaszewicz-Hussain A (2010) Role of oxidative stress in organophosphate insecticide toxicity—short review. Pest Biochem Physiol 98:145–150

Mansour SA, Mossa AH (2010) Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. Pest Biochem Physiol 96:14–23

Miglioranza KSB, Sagrario MDAG, de Moreno JEA, Moreno VJ, Escalante AH, Osterrieth ML (2002) Agricultural soil as a potential source of input of organochlorine pesticides into a nearby pond. Environ Sci Pollut Res 9:250–256

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide. J Biol Chem 247:3170–3175

Mutch E, Williams FM (2007) Diazinon, chlorpyriphos and parathion are metabolized by multiple cytochrome P450 in human liver. Toxicology 224:22–32

Ncibi S, Othman MB, Akacha A, Krifi MN, Zourgui L (2008) *Opuntia ficus indica* extract protects against chlorpyrifos-induced damage on mice liver. Food Chem Toxicol 46:797–802

Nogueira CW, Rocha JBT (2010) Diphenyl diselenide: a janus-faced molecule. J Braz Chem Soc 21:2055–2017

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

Paulmier C (1986) Selenoorganic functional groups. In: Paulmier C (ed) Selenium reagents and intermediates in organic synthesis, 1st edn. Pergamon Press, Oxford, pp 25–51

Prigol M, Luchese C, Nogueira CW (2009a) Antioxidant effect of diphenyl diselenide on oxidative stress caused by acute physical exercise in skeletal muscle and lungs of mice. Cell Biochem Funct 27:216–222

Prigol M, Schumacher RF, Nogueira CW, Zeni G (2009b) Convulsant effect of diphenyl diselenide in rats and mice and its relationship to plasma levels. Toxicol Lett 189:35–39

Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56–63

Reznick AZ, Packer L (1994) Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 233:357–363

Sharma R, Yang Y, Sharma A, Awasthi S, Awastchi YC (2004) Antioxidant role of glutathione *S*-transferases: protection against oxidant toxicity and regulation of stress-mediated apoptosis. Antioxid Redox Signal 6:289–300

Soltatinejad K, Abdollahi M (2009) Current opinion on the science of organophosphate pesticides and toxic stress: a systematic review. Med Sci Monit 15:75–90

Sutcu R, Altuntas I, Yildirim B, Karahan N, Demirin H, Delibas N (2006) The effects of subchronic methidathion toxicity on rat liver: role of antioxidant vitamins C and E. Cell Biol Toxicol 22:221–227

Tripathi S, Srivastav AK (2010) Liver profile of rats after long-term ingestion of different doses of chlorpyrifos. Pest Biochem Physiol 97:60–65

Uzun FG, Demir F, Kalender S, Bas H, Kalender Y (2010) Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. Food Chem Toxicol 48:1714–1720

Verma RS, Mehta A, Srivastava N (2007) In vivo chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins. Pest Biochem Physiol 88:191–196

Wendel A (1981) Glutathione peroxidase. Methods Enzymol 77:325–333

3.2 Artigo 2

O disseleneto de difenila protege contra os distúrbios metabólicos induzidos pela exposição aguda ao acefato em ratos

DIPHENYL DISELENIDE PROTECTS AGAINST METABOLIC DISORDERS INDUCED BY ACEPHATE ACUTE EXPOSURE IN RATS

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Online first na Environmental Toxicology

Diphenyl Diselenide Protects Against Metabolic Disorders Induced by Acephate Acute Exposure in Rats

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Received 21 March 2012; revised 4 June 2012; accepted 10 June 2012

ABSTRACT: The present study investigated the effect of diphenyl diselenide [$(PhSe)_2$] on metabolic disorders induced by acephate acute exposure in rats. We also investigated a possible mechanism of action of $(PhSe)_2$ against hyperglycemia induced by acephate. $(PhSe)_2$ was administered to rats at a dose of 10 or 30 mg/kg by oral gavage (p.o.) 1 hour prior to acephate administration (140 mg/kg; p.o.). Glucose and corticosterone levels as well as the lipid status were determined in plasma of rats. Cardiovascular risk factors and the atherogenic index were calculated. Glycogen levels as well as tyrosine aminotransferase (TAT) and glucose-6-phosphatase (G6Pase) activities were determined in livers of rats. Cerebral acetylcholinesterase (AChE) activity was assayed. Acephate induced an increase in glucose and corticosterone levels as well as in TAT and G6Pase activities. AChE activity was inhibited by acephate. Triglyceride (TG) levels and the cardiovascular risk factor TG/high-density lipoprotein-cholesterol (HDL) were increased by acephate. $(PhSe)_2$ was effective against the metabolic disorders induced by acephate acute exposure in rats.

Keywords: acephate; hyperglycemia; hyperlipidemia; organophosphate; organoselenium; selenium

INTRODUCTION

Organophosphate insecticides (OPs) constitute one of the most widely used classes of pesticides being employed for both agricultural and domestic pest control. The use of OPs has increased considerably due to their low toxicity and low persistence in the mammalian system compared to organochlorine pesticides (Costa, 2006). The wide application of OPs was accompanied by potentially hazardous impact on humans, animals, and environment (water, air, soil, and food), causing severe acute and chronic poisoning (Kanbur et al., 2008; Soltaninejad and Abdollahi, 2009). OPs are primarily recognized for their ability to induce toxicity in mammals through inhibition of acetylcholinesterase (AChE) activity and subsequent activation of cholinergic receptors (Aardema et al., 2008).

Hyperglycemia has been investigated as another facet of OPs toxicity. Studies with animals have shown altered glucose homeostasis following acute and chronic exposure to OPs (Kamath and Rajini, 2007; Lasram et al., 2008). The strength of evidence provided by a systematic review indicates the role of glycogenolysis, gluconeogenesis, and the activation of hypothalamus-pituitary-adrenal (HPA) axis in the mechanisms of OPs-induced hyperglycemia (Rahimi and Abdollahi, 2007). Accordingly, some studies have reported the activation of HPA axis and hepatic gluconeogenesis enzymes, such as tyrosine aminotransferase (TAT) and glucose-6-phosphatase (G6Pase) after OPs exposure in rats (Joshi and Rajini, 2009, 2012).

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Contract grant sponsor: UFSM, CAPES, CNPq, FAPERGS/CNPq (PRONEX)
Contract grant number: 10/0005-1
Contract grant sponsor: FAPERGS
Contract grant number: 10/07116
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/tox.21793

Acephate (*O*,*S*-dimethyl acetylphosphoramidothioate) is an OP widely used in horticulture, household gardens, and for crop protection. Acephate and its primary metabolite, methamidophos, are toxic to various species (Rao et al., 2006). The toxic effects of acephate on experimental animal models, such as neurotoxicity in rats (Chen et al., 2003), genotoxicity in mice (Rahman et al., 2002), and oxidative stress in rats (Datta et al., 2010) have been demonstrated. Furthermore, it was reported that acephate causes a temporary hyperglycemia in rats by activation of the HPA axis and gluconeogenesis pathway (Joshi and Rajini, 2009).

Considering that the exposure to OPs, including acephate, has been related to the development of hyperglycemia, the development of anti-hyperglycemic drugs are of potential interest to reduce hyperglycemia caused by OPs exposure. In this context, the organoselenium compound diphenyl diselenide [$(PhSe)_2$] has numerous pharmacological properties (Nogueira and Rocha, 2010). Of particular importance are the anti-hyperglycemic (Barbosa et al., 2006), anti-diabetic (Barbosa et al., 2008), anti-hyperlipidemic (da Rocha et al., 2011), hepatoprotective (Borges et al., 2008), antiulcer (Savegnago et al., 2006) and antioxidant (Luchese et al., 2007; Prigol et al., 2009) properties demonstrated in different experimental models.

Considering that acephate exposure causes metabolic disorders and $(PhSe)_2$ has anti-hyperglycemic property, the aim of the present study was to investigate the protective effect of $(PhSe)_2$ on metabolic disorders induced by acephate acute exposure in rats. We also investigated a possible mechanism of action of $(PhSe)_2$ against hyperglycemia induced by acephate.

MATERIAL AND METHODS

Chemicals

Acephate (Orthene 750 BR, Arysta Lifescience do Brasil Indústria Química e Agropecuária LTDA) was obtained from commercial grade. The purity of acephate commercial pesticide (74.9%) was determined by gas chromatography with flame ionization detection (GC-FID) according to Dobrat and Martijn (1998). $(PhSe)_2$ was prepared in our laboratory according to Paulmier (1986) and the chemical purity (99.9%) was determined by GC/MS. Analysis of ^1H and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. $(PhSe)_2$ and acephate were dissolved in ethanol and saline, respectively. $(PhSe)_2$ was dissolved in ethanol to avoid a possible interference of another vehicle (oil, for example) in the biochemical determinations related to glucose and lipid metabolism. All other chemicals were obtained from standard commercial suppliers.

Abbreviations

AChE	acetylcholinesterase
AI	atherogenic index
GC-FID	gas chromatography with flame ionization detection
HDL	high-density lipoprotein-cholesterol
HPA	hypothalamus-pituitary-adrenal
LDL	low-density lipoprotein-cholesterol
OPs	organophosphate insecticides
pHBA	*p*-hydroxybenzaldehyde
TAT	tyrosine aminotransferase
TC	total cholesterol
TCA	trichloroacetic acid
TG	triglycerides

Animals

Male adult Wistar rats, weighing 200–300 g, were obtained from our own breeding colony (Federal University of Santa Maria, Brazil). Animals were kept in a separate animal room, on a 12 h light/12 dark cycle with lights on at 7:00 a.m., in an air-conditioned room (22°C ± 2°C). Commercial diet (GUABI, RS, Brasil) and tap water were supplied *ad libitum*. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources and with the approval of the Animal Use Committee (23081.017070/2011-19), Federal University of Santa Maria, Brazil.

Experimental Procedure

Rats were divided into six groups of six animals each. Following overnight fasting (12 hours), $(PhSe)_2$ was administered to rats at a dose of 10 or 30 mg/kg by oral gavage (p.o.) 1 hour prior to p.o. administration of acephate (140 mg/kg of active ingredient). The dose of 10 mg/kg of $(PhSe)_2$, which does not cause toxicity in rodents, was chosen based on our previous study (Barbosa et al., 2008), which demonstrated that $(PhSe)_2$ has anti-hyperglicemic effect on alloxan-induced diabetes in rats. The dose of 30 mg/kg of $(PhSe)_2$ was tested because a dose of 10 mg/kg was not effective against all parameters evaluated in the current study. The dose of 140 mg/kg of acephate was selected based on a previously published study (Joshi and Rajini, 2009), which demonstrated that acephate caused metabolic disorders in rats.

The protocol of rat treatments is given below:

- Group I: ethanol (1 mL/kg; p.o.) plus saline 0.9% (1 mL/kg; p.o.);
- Group II: $(PhSe)_2$ (10 mg/kg; p.o.) plus saline 0.9% (1 mL/kg; p.o.);
- Group III: $(PhSe)_2$ (30 mg/kg; p.o.) plus saline 0.9% (1 mL/kg; p.o.);
- Group IV: ethanol (1 mL/kg; p.o.) plus acephate (140 mg/kg; p.o.);
- Group V: $(PhSe)_2$ (10 mg/kg; p.o.) plus acephate (140 mg/kg; p.o.);
- Group VI: $(PhSe)_2$ (30 mg/kg; p.o.) plus acephate (140 mg/kg; p.o.).

$(PhSe)_2$ was administered at 8:00 a.m. and acephate at 9:00 a.m. Two hours after the acephate administration, all rats were anesthetized for blood collection (2 mL) by heart puncture. Plasma was separated by centrifugation at 2400 × *g* for 10 min and stored at −20°C for biochemical analyzes (hemolyzed plasma was discharged). After this procedure, the rats were killed, and the livers of animals were removed, dissected, and kept on ice until the time of assay. The livers were kept on ice no more than three hours before assay.

The liver samples were homogenized in 50 mM Tris-HCl (pH 7.4; 1:10 w/v) for TAT assay and in 250 mM sucrose containing 1 mM EDTA (pH 7.0; 1:10 w/v) for G6Pase assay and centrifuged at 2400 × *g* for 10 min. The low-speed supernatants (S_1) were separated and used for TAT and G6Pase assays. The brains of rats were removed, kept on ice, homogenized in 0.25 M sucrose buffer (1/20, w/v), and centrifuged at 2400 × *g* for 10 min. The low-speed supernatants (S_1') were used for AChE assay.

Biochemical Determinations

Plasma Glucose Levels Determination

Plasma glucose levels were determined by an enzymatic method based on the oxidase/peroxidase system using a commercial kit (LABTEST, Diagnostica S.A., Minas Gerais, Brasil). Plasma glucose levels were expressed as mg/dL.

Plasma Corticosterone Levels Determination

Corticosterone levels were estimated by the fluorescence method previously described by Zenker and Bernstein (1958). Corticosterone in plasma aliquot (200 μL) was extracted with 2 mL of chloroform. The volume into tubes was completed to 3 mL with distilled water. The tubes were shaken for 15 seconds, centrifuged at 2400 × *g* for 5 minutes, and the aqueous layer was discharged. Then, 1 mL of NaOH 0.1 M was added to tubes which were shaken again for 15 seconds and centrifuged at 2400 × *g* for 5 minutes. The aqueous layer of each tube was discharged as above. Aliquots of 1 mL of chloroform layer were transferred to other tubes containing 3 mL of fluorescence reagent (2.4 parts concentrated H_2SO_4 + 1.0 part 50% ethanol). The tubes were shaken for 15 seconds, centrifuged at 2400 × *g* for 5 minutes, and the chloroform layer was discharged. The tubes with sulfuric acid layer were allowed to stand at room temperature for 2 hours. After that, the fluorescence intensity emission was recorded at 540 nm (with 257 nm excitation). Corticosterone levels were expressed as μg corticosterone/dL plasma.

Plasma Lipid Levels Determination

Total cholesterol (TC), high-density lipoprotein-cholesterol (HDL), and triglycerides (TG) levels were determined by enzymatic colorimetric methods using commercial kits (LABTEST, Diagnostica S.A., Minas Gerais, Brazil). Low-density lipoprotein-cholesterol (LDL) values were obtained by the difference between TC and HDL levels. Plasma lipid levels were expressed as mg/dL.

To explore the lipid metabolism, we calculated the cardiovascular risk factors TC/HDL and TG/HDL and the atherogenic index (AI) [(TC-HDL)/HDL] (Reaven, 2003).

Hepatic Glycogen Levels Determination

The hepatic glycogen content was assayed by the method described by Krisman (1962). Briefly, a known amount of liver was digested in 2 mL of 30% KOH solution. Followed by 10 minutes in boiling water bath, 2 mL of ethanol was added to the tubes to precipitate glycogen. After precipitation, glycogen was resuspended in 0.2 mL 5 M HCl and 0.8 mL distilled water. The glycogen content was measured with iodine reagent at 460 nm and expressed as g of glycogen/100 g of liver.

Hepatic TAT Activity Assay

TAT was assayed by the method described by Diamondstone (1966). The S_1 was diluted in 50 mM Tris-HCl (pH 7.4; 1:2 v/v). The reaction mixture contained 6 mM L-tyrosine, 9.4 mM α-ketoglutarate, 4 mM diethyldithiocarbamate, and 40 μM pyridoxal-5-phosphate in a final volume of 3.2 mL. The reaction was initiated by the addition of 0.025 mL of S_1. The samples were incubated at 37°C for 10 min and the incubation was stopped adding 200 μL of 10 M NaOH. After a stabilization period of 30 min at room temperature, the absorbance of samples was measured at 331 nm. TAT activity was expressed as nmol *p*-hydroxybenzaldehyde (pHBA)/min/mg protein.

Hepatic G6Pase Activity Assay

G6Pase activity was assayed based on the method reported by Ricketts (1963). The S_1 was diluted in 250 mM sucrose containing 1 mM EDTA (pH 7.0; 1:5 v/v) and incubated (0.1 mL) with 50 mM glucose-6-phosphate for 30 minutes at 37°C. The incubation was stopped by adding 1.0 mL of 10% trichloroacetic acid (TCA). The samples were centrifuged at 2400 × *g* for 5 min. Inorganic phosphate (Pi) levels in supernatants were measured at 650 nm as described by Fiske and Subbarow (1925). G6Pase activity was expressed as nmol Pi/min/mg protein.

Cerebral AChE Activity Assay

AChE activity assay was carried out according to the method reported by Ellman et al. (1961), using acetylthiocholine as substrate. The activity of AChE was spectrophotometrically measured at 412 nm and expressed as μmol/h/mg protein.

Protein Determination

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

TABLE I. Effect of $(PhSe)_2$ on biochemical parameters of rats exposed to acephate (AC)

	Experimental Groups					
Parameters	Control	$(PhSe)_2$ (10)	$(PhSe)_2$ (30)	AC	$(PhSe)_2$ (10) + AC	$(PhSe)_2$ (30) + AC
Glucose[a]	91.46 ± 4.25	93.11 ± 7.26	104.30 ± 7.01	229.30 ± 12.88*	132.20 ± 11.78*#	121.80 ± 9.59*#
G6Pase[b]	38.50 ± 1.88	42.13 ± 1.40	37.47 ± 1.64	64.22 ± 3.47*	54.55 ± 2.54*#	46.53 ± 4.68#
TAT[c]	18.60 ± 1.16	21.35 ± 1.05	17.42 ± 1.37	31.84 ± 1.48*	32.36 ± 2.76*	26.02 ± 1.65*#
Corticosterone[d]	29.92 ± 2.47	31.63 ± 3.52	34.47 ± 0.67	56.33 ± 3.41*	52.10 ± 1.05*	52.97 ± 1.82*
AChE[e]	6.25 ± 0.30	5.81 ± 0.30	7.07 ± 0.85	2.50 ± 0.27*	3.31 ± 0.63*	2.46 ± 0.26*
TC[a]	90.50 ± 8.16	90.17 ± 10.33	75.00 ± 4.81	86.83 ± 8.22	88.50 ± 5.24	72.83 ± 8.37
HDL[a]	42.00 ± 3.87	37.00 ± 2.54	34.00 ± 2.37	37.17 ± 3.72	37.67 ± 1.84	37.50 ± 2.22
LDL[a]	37.90 ± 5.65	43.27 ± 7.86	33.47 ± 5.17	38.83 ± 5.38	41.43 ± 3.90	29.60 ± 6.63
TG[a]	44.67 ± 3.40	49.83 ± 4.59	37.67 ± 2.90	62.50 ± 4.17*	47.00 ± 2.87#	48.67 ± 1.20#
TG/HDL	1.13 ± 0.16	1.35 ± 0.10	1.12 ± 0.08	1.76 ± 0.19*	1.27 ± 0.12#	1.31 ± 0.07#

[a] Plasma glucose, TC, HDL, LDL and TG levels are expressed as mg/dL.
[b] Hepatic G6Pase activity is expressed as nmol Pi/min/mg protein.
[c] Hepatic TAT activity is expressed as nmol pHBA/min/mg protein.
[d] Plasma corticosterone levels are expressed as μg corticosterone/dL plasma.
[e] Cerebral AChE activity is expressed as μmol/h/mg protein.
* Denotes $p < 0.05$ as compared to the control group.
# Denotes $p < 0.05$ as compared to the AC group (two-way ANOVA/Duncan's multiple range test).
Data are reported as means ± S.E.M. of six animals per group.

Statistical Analysis

Statistical analysis was performed using a two-way analysis of variance (ANOVA), followed by the Duncan's Multiple Range Test. Main effects are presented only when the higher second-order interaction was nonsignificant. Data were expressed as means ± S.E.M. of six animals. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Plasma Glucose Levels

As shown in Table I, plasma glucose levels in control rats were 91.46 ± 4.25 mg/dL. Acephate-exposed rats presented glucose levels of 229.30 ± 12.88 mg/dL while in acephate-exposed rats pre-treated with $(PhSe)_2$ (10 or 30 mg/kg) glucose levels were 132.20 ± 11.78 and 121.80 ± 9.59 mg/dL, respectively. The two-way ANOVA of glucose levels demonstrated a significant $(PhSe)_2$ × acephate interaction ($F_{2,20}$= 44.51, $p < 0.0001$). Post-hoc comparisons revealed that acephate increased glucose levels of rats if compared to those of the control group. $(PhSe)_2$ pretreatment at doses of 10 and 30 mg/kg attenuated the increase of glucose levels caused by acephate.

$(PhSe)_2$ at doses of 10 and 30 mg/kg did not alter glucose levels in plasma of rats (93.11 ± 7.26 and 104.30 ± 7.01 mg/dL, respectively) (Table I).

Hepatic Glycogen Levels

The two-way ANOVA showed that neither $(PhSe)_2$ nor acephate administration alter glycogen levels in livers of rats (data not shown).

Hepatic G6Pase Activity

Hepatic G6Pase activity in control rats was 38.50 ± 1.88 nmol Pi/min/mg protein. Acephate-exposed rats presented G6Pase activity of 64.22 ± 3.47 nmol Pi/min/mg protein, whereas in acephate-exposed rats pretreated with $(PhSe)_2$ (10 or 30 mg/kg) G6Pase activity were 54.55 ± 2.54 and 46.53 ± 4.68 nmol Pi/min/mg protein, respectively. The two-way ANOVA of G6Pase activity yielded a significant $(PhSe)_2$ x acephate interaction ($F_{2,20}$ = 6.91, $p < 0.05$). Post-hoc comparisons showed that acephate increased G6Pase activity if compared to that of rats from the control group. $(PhSe)_2$ pretreatment at doses of 10 and 30 mg/kg partially and completely protected against the increase of G6Pase activity caused by acephate, respectively (Table I).

$(PhSe)_2$ at doses of 10 and 30 mg/kg did not alter G6Pase activity in livers of rats (42.13 ± 1.40 and 37.47 ± 1.64 nmol Pi/min/mg protein, respectively) (Table I).

Hepatic TAT Activity

Hepatic TAT activity in control rats was 18.60 ± 1.16 nmol pHBA/min/mg protein. Acephate-exposed rats presented TAT activity of 31.84 ± 1.48 nmol pHBA/min/mg protein while in acephate-exposed rats pretreated with $(PhSe)_2$ (10 or 30 mg/kg) TAT activity were 32.36 ± 2.76 and 26.02 ± 1.65 nmol pHBA/min/mg protein, respectively. The two-way ANOVA of TAT activity revealed a significant main effect of acephate ($F_{1,20}$ = 58.40, $p < 0.0001$) and $(PhSe)_2$ ($F_{2,20}$= 5.99, $p < 0.05$). Post-hoc comparisons demonstrated that acephate increased TAT activity when compared to the control group. $(PhSe)_2$ pretreatment at a dose of 30 mg/kg partially protected against

the increase of TAT activity resulting from acephate exposure (Table I).

Hepatic TAT was not altered by $(PhSe)_2$ pretreatment at doses of 10 mg/kg and 30 mg/kg (21.35 ± 1.05 and 17.42 ± 1.37 nmol pHBA/min/mg protein, respectively) (Table I).

Plasma Corticosterone Levels

As shown in Table I, plasma corticosterone levels in control rats were 29.92 ± 2.47 μg corticosterone/dL plasma. Acephate-exposed rats presented corticosterone levels of 56.33 ± 3.41 μg corticosterone/dL plasma while in acephate-exposed rats pretreated with $(PhSe)_2$ (10 or 30 mg/kg) corticosterone levels were 52.10 ± 1.05 and 52.97 ± 1.82 μg corticosterone/dL plasma, respectively. The two-way ANOVA of corticosterone levels data showed a significant main effect of acephate ($F_{1,20}$= 93.62, $p < 0.0001$). Post-hoc comparisons revealed that acephate increased corticosterone levels when compared to those of rats from the control group. $(PhSe)_2$ pretreatment at both doses did not protect against the increase of corticosterone levels caused by acephate.

Corticosterone levels remained unaltered in plasma of rats which received $(PhSe)_2$ at doses of 10 and 30 mg/kg (31.63 ± 3.52 and 34.47 ± 0.67 μg corticosterone/dl plasma, respectively) (Table I).

Cerebral AChE Activity

Cerebral AChE activity in control rats was 6.25 ± 0.30 μmol/h/mg protein. Acephate-exposed rats presented AChE activity of 2.50 ± 0.27 μmol/h/mg protein, while in acephate-exposed rats pretreated with $(PhSe)_2$ (10 or 30 mg/kg) AChE activity were 3.31 ± 0.63 and 2.46 ± 0.26 μmol/h/mg protein, respectively. The two-way ANOVA of AChE activity yielded a significant main effect of acephate ($F_{1,20}$= 72.81, $p < 0.0001$). Post-hoc comparisons demonstrated that acephate inhibited AChE activity in brains of rats if compared to those of the control group. $(PhSe)_2$ pretreatment at both doses did not protect against the inhibition of AChE activity resulting from acephate exposure (Table I).

$(PhSe)_2$ at doses of 10 and 30 mg/kg did not alter AChE activity in brains of rats (5.81 ± 0.30 and 7.07 ± 0.85 μmol/h/mg protein, respectively) (Table I).

Plasma Lipid Status

TC, HDL, and LDL levels were not altered in plasma of rats administered with $(PhSe)_2$ and/or acephate (Table I).

Plasma TG levels in control rats were 44.67 ± 3.40 mg/dL. Acephate-exposed rats presented TG levels of 62.50 ± 4.17 mg/dL, whereas in acephate-exposed rats pretreated with $(PhSe)_2$ (10 or 30 mg/kg) TG levels were 47.00 ± 2.87 and 48.67 ± 1.20 mg/dL, respectively. The two-way ANOVA of TG levels demonstrated a significant $(PhSe)_2$ × acephate interaction ($F_{2,20}$= 7.32, $p < 0.05$). Post-hoc comparisons revealed that acephate increased TG levels of rats if compared to those of the control group. $(PhSe)_2$ pretreatment at doses of 10 and 30 mg/kg completely protected against the increase of TG levels caused by acephate (Table I).

$(PhSe)_2$ at doses of 10 and 30 mg/kg did not alter TG levels in plasma of rats (49.83 ± 4.59 and 37.67 ± 2.90 mg/dL, respectively) (Table I).

The cardiovascular risk factor TG/HDL in control rats was 1.13 ± 0.16. Acephate-exposed rats presented TG/HDL of 1.76 ± 0.19, while in acephate-exposed rats pretreated with both doses of $(PhSe)_2$ TG/HDL were 1.27 ± 0.12 and 1.31 ± 0.07. The two-way ANOVA of the cardiovascular risk factor TG/HDL data yielded a significant $(PhSe)_2$ × acephate interaction ($F_{2,20}$= 5.96, $p < 0.05$). Post-hoc comparisons showed that acephate increased TG/HDL ratio when compared to the control group. $(PhSe)_2$ pretreatment at doses of 10 and 30 mg/kg was effective against the increase of TG/HDL ratio caused by acephate (Table I).

$(PhSe)_2$ at doses of 10 and 30 mg/kg did not alter TG/HDL ratio of rats (1.35 ± 0.10 and 1.12 ± 0.08, respectively) (Table I).

The cardiovascular risk factor TC/HDL and the atherogenic index were not altered in rats administered with $(PhSe)_2$ and/or acephate (data not shown).

DISCUSSION

In the current study, we reported the protective effect of $(PhSe)_2$ on metabolic disorders induced by acephate acute exposure in rats. Acephate acute exposure induced hyperglycemia in rats, which was demonstrated by increased glucose levels in plasma. Furthermore, acephate caused an increase of corticosterone levels and hepatic TAT and G6Pase activities, associated with an inhibition of cerebral AChE activity. In addition, acephate induced an increase in plasma TG levels and of the cardiovascular risk factor TG/HDL in rats. $(PhSe)_2$ attenuated these alterations induced by acephate, except for the increase of corticosterone levels and AChE activity inhibition.

With agreement with Joshi and Rajini (2009) in our study, we demonstrated the hyperglycemic effect of acephate after 2 hours of a single acute administration in rats. $(PhSe)_2$ attenuated the increase of glucose levels caused by acephate, indicating its anti-hyperglycemic property. Accordingly, similar effect of $(PhSe)_2$ was reported when this compound was administered to diabetic rats either induced by alloxan or streptozotocin (Barbosa et al., 2008; Kade et al., 2009).

Our results showed that acephate stimulates the HPA axis as demonstrated by the increase of corticosterone levels in plasma of rats. Similarly, other authors have demonstrated this effect after acephate and monocrotophos administration in rats (Spassova et al., 2000; Joshi and Rajini, 2009; 2012). Moreover, the activation of HPA axis seems to be related to the inhibition of cerebral AChE activity since the accumulation of ACh stimulates the hypothalamus to release the corticotropin-releasing hormone (Bugajski et al., 2001). In the present study, $(PhSe)_2$ was not effective against the AChE activity inhibition and consequently did not protect against the increase of corticosterone levels. These results suggest that the modulation of the HPA axis is not involved in the anti-hyperglycemic effect of $(PhSe)_2$.

Besides to the activation of HPA axis, our results showed increased TAT and G6Pase activities in livers of rats exposed to acephate. These results probably are a consequence of the increase of corticosterone levels. $(PhSe)_2$ at a dose of 30 mg/kg partially protected against the increase of TAT activity and completely protected against the increase of G6Pase. These results indicate that the modulation of the gluconeogenic enzyme activities is one of the mechanisms involved in the anti-hyperglycemic effect of $(PhSe)_2$.

However, one cannot rule out the involvement of other mechanisms in the anti-hyperglycemic effect of $(PhSe)_2$. In fact, in the current study we found that $(PhSe)_2$ at doses of 10 and 30 mg/kg similarly attenuated the rise in glucose levels and differently protected against the increase of TAT and G6Pase activities resulting from acephate exposure. $(PhSe)_2$ at a dose of 10 mg/kg did not protect against the increase of TAT activity and partially protected against the increase of G6Pase activity. Conversely, at a dose of 30 mg/kg $(PhSe)_2$ partially and completely protected against the increase of TAT and G6Pase activities, respectively. Taken these results collectively one can suggest that other mechanisms, besides the modulation of the activity of these gluconeogenic enzymes, may be involved in the anti-hyperglycemic effect of $(PhSe)_2$. However, these mechanisms remain to be elucidated.

Furthermore, in the present study acephate caused a significant increase in plasma TG levels and of the cardiovascular risk factor TG/HDL. $(PhSe)_2$ completely protected against the increase of plasma TG levels and of the TG/HDL ratio demonstrating its anti-hyperlipidemic and cardioprotective activity. Accordingly, the anti-hyperlipidemic property of $(PhSe)_2$ was demonstrated in other experimental protocols (da Rocha et al., 2009, 2011). However, the exact mechanism by which $(PhSe)_2$ acts as an anti-hyperlipidemic drug remains to be elucidated.

In conclusion, this study demonstrated that pretreatment with $(PhSe)_2$ was effective against metabolic disorders induced by acephate acute exposure in rats. Further studies are needed to elucidate the exact mechanism by which $(PhSe)_2$ exerted its anti-hyperglycemic and anti-hyperlipidemic properties, since this organoselemium compound could be a promising alternative to minimize metabolic disorders associated with OPs exposure.

REFERENCES

Aardema H, Meertens JHJM, Ligtenberg JJM, Peters-Polman OM, Tulleken JE, Zijlstra JG. 2008. Organophosphorus pesticide poisoning: Cases and developments. Neth J Med 66:149–153.

Barbosa NBV, Rocha JBT, Wondracek DC, Perottoni J, Zeni G, Nogueira CW. 2006. Diphenyl diselenide reduces temporarily hyperglycemia: Possible relationship with oxidative stress. Chem Biol Interact 163:230–238.

Barbosa NBV, Oliveira C, Araldi D, Folmer V, Rocha JBT, Nogueira CW. 2008. Acute diphenyl diselenide treatment reduces hyperglycemia but does not change delta-aminolevulinate dehydratase activity in alloxan-induced diabetes in rats. Biol Pharm Bull 31:2200–2204.

Borges LP, Brandao R, Godoi B, Nogueira CW, Zeni G. 2008. Oral administration of diphenyl diselenide protects against cadmium-induced liver damage in rats. Chem Biol Interact 171:15–25.

Bradford MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principles of protein-dye binding. Anal Biochem 72:248–254.

Bugajski J, Gadek-Michalska A, Bugajski AJ. 2001. A single corticosterone pretreatment inhibits the hypothalamic-pituitary-adrenal responses to adrenergic and cholinergic stimulation. J Physiol Pharmacol 52:313–324.

Chen D, Shi N, Li T, Wang B. 2003. Comparative study of the toxic effects of methamidophos and acephate on intracellular free Ca^{2+} and cAMP concentrations in rat brain tissue. Toxicology 191:34–35.

Costa LG. 2006. Current issues in organophosphate toxicology. Clin Chim Acta 366:1–13.

Da Rocha JT, Sperança A, Nogueira CW, Zeni G. 2009. Hypolipidaemic activity of orally administered diphenyl diselenide in Triton WR-1339-induced hyperlipidaemia in mice. J Pharm Pharmacol 61:1673–1679.

Da Rocha JT, Pinton S, Mazzanti A, Mazzanti CM, Beckemann DV, Nogueira CW, Zeni G. 2011. Effects of diphenyl diselenide on lipid profile and hepatic oxidative stress parameters in ovariectomized female rats. J Pharm Pharmacol 63:663–669.

Datta S, Dhar P, Mukherjee A, Ghosh S. 2010. Influence of polyphenolic extracts from *Enydra fluctuans* on oxidative stress induced by acephate in rats. Food Chem Toxicol 48:2766–2771.

Diamondstone TI. 1966. Assay of tyrosine transaminase activity by conversion of p-hydroxyphenylpyruvate to p-hydroxybenzaldehyde. Anal Biochem 16:395–401.

Dobrat W, Martijn A (editors). 1998. CIPAC Handbook Volume H: Analysis of Technical and Formulated Pesticides. Cambridge, UK: Black Bear Press. p395.

Ellman GL, Courtney KD, Andres V, Featherstone RM. 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95.

Fiske CH, Subbarow YJ. 1925. The colorimetric determination of phosphorus. Biol Chem 66:375–381.

Joshi AKR, Rajini PS. 2009. Reversible hyperglycemia in rats following acute exposure to acephate, an organophosphorus insecticide: Role of gluconeogenesis. Toxicology 257:40–45.

Joshi AKR, Rajini PS. 2012. Hyperglycemic and stressogenic effects of monocrotophos in rats: Evidence for the involvement of acetylcholinesterase inhibition. Exp Toxicol Pathol 64:115–120.

Kade IJ, Borges VC, Savegnago L, Ibukun EO, Zeni G, Nogueira CW, Rocha JBT. 2009. Effect of oral administration of diphenyl diselenide on antioxidant status, and activity of delta aminolevulinic acid dehydratase and isoforms of lactate dehydrogenase, in streptozotocin-induced diabetic rats. Cell Biol Toxicol 25:415–424.

Kamath V, Rajini PS. 2007. Altered glucose homeostasis and oxidative impairment in pancreas of rat subjected to dimethoate intoxication. Toxicology 231:137–146.

Kanbur M, Liman BC, Eraslan G, Altinordulu S. 2008. Effects of cypermethrin, propetamphos, and combination involving cypermethrin and propetamphos on lipid peroxidation in mice. Environ Toxicol 23:473–479.

Krisman CR. 1962. A method for the colorimetric estimation of glycogen with iodine. Anal Biochem 4:17–23.

Lasram MM, Annabi AB, Rezg R, Elj N, Slimen S, Kamoun A, El-Fazaa S, Gharbi N. 2008. Effect of short-time malathion administration on glucose homeostasis in Wistar rat. Pestic Biochem Physiol 92:114–119.

Luchese C, Brandão R, de Oliveira R, Nogueira CW, Santos FW. 2007. Efficacy of diphenyl diselenide against cerebral and pulmonary damage induced by cadmium in mice. Toxicol Lett 173:181–190.

Nogueira CW, Rocha JBT. 2010. Diphenyl diselenide: A janus-faced molecule. J Braz Chem Soc 21:2055–2017.

Paulmier C. 1986. Selenoorganic functional groups. In: Paulmier C, editor. Selenium Reagents and Intermediates in Organic Synthesis. Oxford: Pergamon Press.1st ed. pp 25–51.

Prigol M, Luchese C, Nogueira CW. 2009. Antioxidant effect of diphenyl diselenide on oxidative stress caused by acute physical exercise in skeletal muscle and lungs of mice. Cell Biochem Funct 27:216–222.

Rahimi R, Abdollahi M. 2007. A review on mechanisms involved in hyperglycemia induced by organophosphorus insecticides. Pest Biochem Physiol 88:115–121.

Rahman MF, Mahboob M, Danadevi K, Saleha Banu B, Grover P. 2002. Assessment of genotoxic effects of chloropyriphos and acephate by the comet assay in mice leucocytes. Mutat Res 516:139–147.

Rao JV, Srikanth K, Arepalli SK, Gunda VG. 2006. Toxic effects of acephate on *Paramecium caudatum* with special emphasis on morphology, behaviour, and generation time. Pestic Biochem Physiol 86:131–137.

Reaven GM. 2003. Importance of identifying the overweight patient who will benefit the most by losing weight. Ann Intern Med 138:420–423.

Ricketts TR. 1963. An improved micromethod for the determination of glucose-6 phosphatase activity. Clin Chim Acta 8:160–162.

Savegnago L, Trevisan M, Alves D, Rocha JBT, Nogueira CW, Zeni G. 2006. Antisecretory and antiulcer effects of diphenyl diselenide. Environ Toxicol Pharmacol 21:86–92.

Soltaninejad K, Abdollahi M. 2009. Current opinion on the science of organophosphate pesticides and toxic stress: A systematic review. Med Sci Monit 15:75–90.

Spassova D, White T, Singh AK. 2000. Acute effects of acephate and methamidophos on acetylcholinesterase activity, endocrine system, and amino acid concentrations in rats. Comp Biochem Physiol C 126:79–89.

Zenker N, Bernstein DE. 1958. The estimation of small amounts of corticosterone in rat plasma. J Biol Chem 231:695–701.

3.3 Artigo 3

A exposição aguda ao clorpirifós induz hiperglicemia e hiperlipidemia em ratos

CHLORPYRIFOS ACUTE EXPOSURE INDUCES HYPERGLYCEMIA AND HYPERLIPIDEMIA IN RATS

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Chemosphere 89 (2012) 602-608

Chemosphere 89 (2012) 602–608

Contents lists available at SciVerse ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Chlorpyrifos acute exposure induces hyperglycemia and hyperlipidemia in rats

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HIGHLIGHTS

- Hyperglycemic and hyperlipidemic effects of chlorpyrifos in rats were investigated.
- A single chlorpyrifos administration caused hyperglycemia and hyperlipidemia in rats.
- Chlorpyrifos increased corticosterone levels and gluconeogenic enzyme activities.
- Gluconeogenesis pathway is involved in the hyperglycemic effect of chlorpyrifos.
- Chlorpyrifos increased triglycerides and low-density lipoprotein-cholesterol levels.

ARTICLE INFO

Article history:
Received 29 November 2011
Received in revised form 24 January 2012
Accepted 16 May 2012
Available online 23 July 2012

Keywords:
Pesticides
Organophosphate
Chlorpyrifos
Hyperglycemia
Hyperlipidemia
Rats

ABSTRACT

In this study we evaluated the hyperglycemic and hyperlipidemic effects of chlorpyrifos (CPF) after an acute exposure in rats. The mechanisms involved in hyperglycemia induced by CPF were studied. A single dose of CPF (50 mg kg^{-1}, subcutaneous, s.c.) was administered to overnight-fasted rats. Glucose and corticosterone levels, lipid status and paraoxonase (PON1) activity were determined in plasma of rats. Cardiovascular risk factors and the atherogenic index were calculated. Glycogen levels, tyrosine aminotransferase (TAT) and glucose-6-phosphatase (G6Pase) activities were determined in livers of rats. Cerebral acetylcholinesterase (AChE) activity was also determined. CPF caused an increase in glucose and glycogen levels as well as in TAT and G6Pase activities. The CPF exposure caused an increase in corticosterone levels, an inhibition of AChE activity and a reduction of PON1 activity. Regarding the lipid status, CPF induced an increase in triglycerides (TG) and low-density lipoprotein-cholesterol (LDL) levels and a decrease in high-density lipoprotein (HDL) levels associated with an increase of cardiovascular risk factors and the atherogenic index. The present study demonstrated that a single CPF administration caused hyperglycemia and hyperlipidemia in rats. The activation of the gluconeogenesis pathway, probably elicited by hypercorticosteronemia, is involved in the hyperglycemic effect of CPF in rats.

1. Introduction

The widespread use of organophosphate insecticides (OPs) in public health and agricultural programs has caused severe environmental pollution and potential health hazards including acute and chronic cases of human poisoning (Soltaninejad and Abdollahi, 2009). OPs have been reported to exert their primary toxic effects by phosphorylating the serine residue at the active site of acetylcholinesterase (AChE), and thus inhibiting this enzyme. AChE is responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh) to choline and acetate during neurotransmission (Kwong, 2002). In addition to cause neurotoxicity, OPs are also related to a variety of physiological abnormalities including immunotoxicity (Galloway and Handy, 2003), oxidative stress (Kamath et al., 2008; Wu et al., 2011), alterations in glucose homeostasis (Kamath and Rajini, 2007) and hyperglycemia (Joshi and Rajini, 2009).

Among the various toxic effects caused by OPs, the mechanisms affecting glucose homeostasis have been under investigation in the recent years. Stimulation of hepatic gluconeogenesis and glycogenolysis is proposed as some of underlying mechanisms of OPs-induced hyperglycemia (Abdollahi et al., 2004). Another proposed mechanism of OPs-induced hyperglycemia is the activation of the hypothalamus–pituitary–adrenal (HPA) axis. The activation of HPA axis by OPs causes secretion of glucocorticoids from adrenal cortex that in turn increases blood glucose by induction of gluconeogenesis pathway (Rahimi and Abdollahi, 2007). In fact, some studies have reported the coexistence of hyperglycemia, activation of HPA axis and increased activities of hepatic gluconeogenesis enzymes, such as tyrosine aminotransferase (TAT) and glucose-6-phosphatase (G6Pase) after OPs acute exposure (Joshi and Rajini, 2009, 2012).

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http://dx.doi.org/10.1016/j.chemosphere.2012.05.059

In addition to the hyperglycemic effect induced by OPs, some studies have shown that these compounds can also induce a disturbance in the lipid status, such as an increase of cholesterol and triglycerides levels that represents a risk factor for premature atherosclerosis (Çetin et al., 2010; Lasram et al., 2009). However, there is little information in the literature concerning the mechanisms involved in hyperlipidemia induced by OPs.

Chlorpyrifos (CPF) is a broad spectrum OP widely used for a variety of agricultural and public health applications (Rusyniak and Nanagas, 2004). It is metabolically activated in liver to its corresponding oxygen analog, CPF-oxon, which is primarily responsible for the mammalian toxicity (Kousba et al., 2004). CPF-oxon can be detoxified by hepatic and extra-hepatic esterases, such as paraoxonase (PON1) (Busby-Hjerpe et al., 2010). Several toxic effects have been reported after acute or chronic exposure to CPF in experimental animals, such as hepatotoxicity and nephrotoxicity (Verma and Srivastava, 2003), teratogenicity (Tian et al., 2005), genotoxicity (Mehta et al., 2008), developmental toxicity (Chen et al., 2011) among others. However, there are no reports regarding the effect of CPF acute exposure on the glucose homeostasis and lipid status in rats.

In view of the above considerations, the present investigation was carried out to (i) investigate the hyperglycemic and hyperlipidemic effects of CPF following an acute exposure in rats; (ii) elucidate the mechanisms involved in hyperglycemia induced by CPF in rats.

2. Materials and methods

2.1. Chemicals

CPF (La Forja S.A.) was obtained from commercial grade. The purity of CPF commercial pesticide (47.2%) was determined by gas chromatography with flame ionization detection (GC-FID) according to Dobrat and Martijn (1998). CPF was dissolved in saline. All other chemicals were obtained from standard commercial suppliers.

2.2. Animals

Male adult Wistar rats, weighing 200–300 g, were obtained from a local breeding colony. Animals were kept in a separate animal room, on a 12 h light/12 dark cycle with lights on at 7:00 a.m., in an air-conditioned room (22 ± 2 °C). Commercial diet (GUABI, RS, Brasil) and tap water were supplied *ad libitum*. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources and with the approval of the Animal Use Committee (23081.017070/2011-19), Federal University of Santa Maria, Brasil.

2.3. Experimental procedure

A single dose of CPF (50 mg kg^{-1} of the active ingredient, subcutaneous, s.c.) was administered to overnight-fasted rats. The control groups received equal amount of saline (1 mL kg^{-1}). The dose of CPF was chosen based on a pilot study performed by our research group.

The animals were observed for signs of CPF toxicity (as salivation, tremors, death). At the end of CPF exposure, rats were anesthetized for blood collection by heart puncture and killed at 2, 4, 8, 12 and 24 h after CPF administration. Plasma was separated by centrifugation at 2400*g* for 10 min and stored at −20 °C for biochemical analyzes (hemolyzed plasma was discharged). The livers of animals were removed, dissected and kept on ice until the time of assay.

The liver samples were homogenized in 50 mM Tris-HCl (pH 7.4; 1:10 w/v) for TAT assay and in 250 mM sucrose containing 1 mM EDTA (pH 7.0; 1:10 w/v) for G6Pase assay and centrifuged at 2400*g* for 10 min. The low-speed supernatants (S_1) were separated and used for TAT and G6Pase analyzes.

The brains of rats were removed, kept on ice, homogenized in 0.25 M sucrose buffer (1/20, w/v) and centrifuged at 2400*g* for 10 min. The low-speed supernatants (S_1') were used for AChE assay.

2.4. Biochemical determinations

2.4.1. Plasma glucose levels

Plasma glucose levels were determined by an enzymatic method based on the oxidase/peroxidase system using a commercial kit (LABTEST, Diagnostica S.A., Minas Gerais, Brasil). Plasma glucose levels were expressed as mg dL^{-1}.

2.4.2. Plasma lipid levels

Total cholesterol (TC), high-density lipoprotein-cholesterol (HDL) and triglycerides (TG) levels were determined by enzymatic colorimetric methods using commercial kits (LABTEST, Diagnostica S.A., Minas Gerais, Brazil). Low-density lipoprotein-cholesterol (LDL) values were obtained by the difference between TC and HDL levels. Plasma lipid levels were expressed as mg dL^{-1}.

To explore the lipid metabolism, we calculated cardiovascular risk factors TC/HDL and TG/HDL and the atherogenic index (AI) [(TC–HDL)/HDL] (Reaven, 2003).

2.4.3. Plasma corticosterone levels

Corticosterone levels were determined by the fluorescence method previously described by Zenker and Bernstein (1958). Corticosterone in plasma aliquot (200 μL) was extracted with 2 mL of chloroform. The volume into tubes was completed to 3 mL with distilled water. The tubes were shaken for 15 s, centrifuged at 2400*g* for 5 min and the aqueous layer was discharged. Then, 1 mL of NaOH 0.1 M was added to tubes which were shaken again for 15 s and centrifuged at 2400*g* for 5 min. The aqueous layer of each tube was discharged as above. Aliquots of 1 mL of chloroform layer were transferred to other tubes containing 3 mL of fluorescence reagent (2.4 parts concentrated H_2SO_4 + 1.0 part 50% ethanol). The tubes were shaken for 15 s, centrifuged at 2400*g* for 5 min and the chloroform layer was discharged. The tubes with sulfuric acid layer were allowed to stand at room temperature for 2 h. After that, the fluorescence intensity emission was recorded at 540 nm (with 257 nm excitation). Corticosterone levels were expressed as μg corticosterone dL^{-1}.

2.4.4. Plasma PON1 activity

PON1 activity was determined in plasma of rats (Ayub et al., 1999) by measuring the initial rate of paraoxon hydrolysis to yield *p*-nitrophenol at 412 nm and 25 °C. The absorbance was monitored in the assay mixture (800 μL) containing 2.0 mM paraoxon, 2.0 mM $CaCl_2$ and 50 μL of plasma in 100 mM Tris–HCl buffer (pH 8.0). The blank sample containing the incubation mixture without plasma was run to correct for spontaneous substrate breakdown. The enzyme activity was calculated from the molar absorption coefficient of *p*-nitrophenol ($18.29 \times 10^3\ M^{-1}\ cm^{-1}$) and was expressed as U mL^{-1} (1U of enzyme hydrolyses 1 nmol of paraoxon min^{-1}).

2.4.5. Hepatic glycogen levels

Hepatic glycogen content was determined by the method described by Krisman (1962). Briefly, a known amount of liver was digested in 2 mL of 30% KOH solution. Followed by 10 min in boiling water bath, 2 mL of ethanol was added to the tubes to precipitate glycogen. After precipitation, glycogen was resuspended in 0.2 mL 5 M HCl and 0.8 mL distilled water. The glycogen content

was measured with iodine reagent at 460 nm and expressed as g of glycogen 100 g of liver^{-1}.

2.4.6. Hepatic TAT activity

TAT was assayed by the method described by Diamondstone (1966). The S_1 was diluted in 50 mM Tris–HCl (pH 7.4; 1:2 v/v). The reaction mixture contained 6 mM L-tyrosine, 9.4 mM α-ketoglutarate, 4 mM diethyldithiocarbamate and 40 μM pyridoxal-5-phosphate, in a final volume of 3.2 mL. The reaction was initiated by the addition of 0.025 mL of S_1. The samples were incubated at 37 °C for 10 min and the incubation was stopped adding 200 μL of 10 M NaOH. After a stabilization period of 30 min at room temperature, the absorbance of samples was measured at 331 nm. TAT activity was expressed as nmol p-hydroxybenzaldehyde (pHBA) min^{-1} mg protein^{-1}.

2.4.7. Hepatic G6Pase activity

G6Pase activity was determined based on the method described by Ricketts (1963). The S_1 was diluted in 250 mM sucrose containing 1 mM EDTA (pH 7.0; 1:5 v/v) and incubated (0.1 mL) with 50 mM glucose-6-phosphate for 30 min at 37 °C. The incubation was stopped by adding 1.0 mL of 10% trichloroacetic acid (TCA). The samples were centrifuged at 2400*g* for 5 min. Inorganic phosphate (Pi) levels in supernatants were measured at 650 nm as described by Fiske and Subbarow (1925). G6Pase activity was expressed as nmol Pi min^{-1} mg protein^{-1}.

2.4.8. Cerebral AChE activity

AChE activity assay was carried out according to the method reported by Ellman et al. (1961), using acetylthiocholine as substrate. The activity of AChE was spectrophotometrically measured at 412 nm and expressed as μmol h^{-1} mg protein^{-1}.

2.4.9. Protein determination

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

2.5. Statistical analysis

Data are expressed as the mean (s) ± S.E.M. of eight animals. Statistical analysis was performed comparing the CPF group with the control group of the corresponding exposure time using unpaired Student's *t*-test. A value of $p < 0.05$ was considered to be statistically significant. Pearson's correlation coefficient was used for the estimation of correlation between parameters analyzed. For the correlation analysis, results from all animals over all time periods were used.

3. Results

3.1. Signs of toxicity

No death was observed in CPF-exposed animals until the end of the experiment. Signs of cholinergic toxicity (as tremors, weakness and diarrhea) were observed only in animals exposed for 24 h to CPF.

3.2. Glucose levels

The Student's *t*-test of glucose levels data demonstrated that CPF induced an increase in plasma glucose levels after 8–24 h of exposure, reaching a maximum increase (89% over control; $p < 0.0001$) at 24 h. Glucose levels measured at 8 and 12 h after CPF administration were 45% ($p < 0.0001$) and 43% ($p = 0.0071$) higher than those of the corresponding control rats, respectively (Fig. 1A).

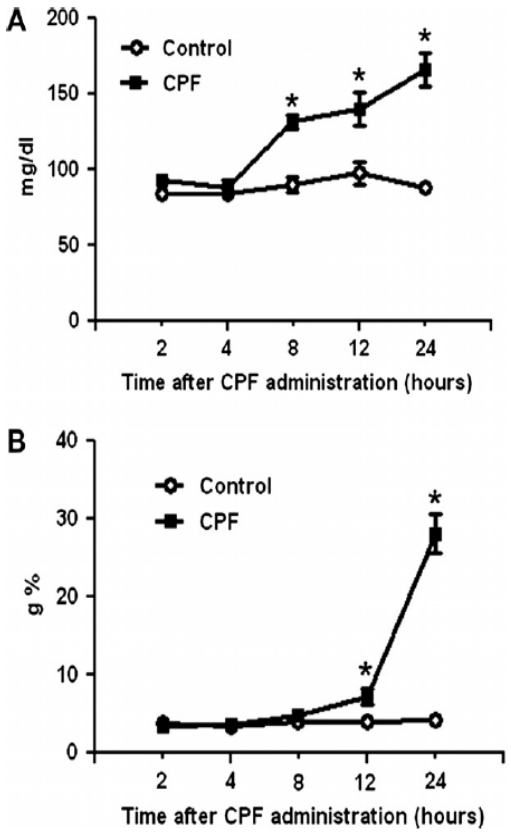

Fig. 1. Effect of CPF acute administration (50 mg kg^{-1}; s.c.) on plasma glucose (A) and hepatic glycogen (B) levels in rats. Data are reported as the mean (s) ± S.E.M. of eight animals per group and expressed as mg dL^{-1} and g%, respectively. (*) Denotes $p < 0.05$ as compared to the control group of the corresponding exposure time (unpaired Student's *t*-test).

3.3. Glycogen levels

The Student's *t*-test showed that CPF induced a marked increase of glycogen levels in livers of rats after 12 ($p = 0.0046$) and 24 h ($p < 0.0001$) of exposure when compared to those of the corresponding control groups. The increase of hepatic glycogen levels were about 82% and 577% (sixfold) after 12 and 24 h of the CPF administration, respectively (Fig. 1B).

3.4. TAT activity

The Student's *t*-test of TAT activity revealed that CPF increased the enzyme activity in livers of rats after 12 ($p = 0.0006$) and 24 h ($p < 0.0001$) of exposure when compared to the corresponding control groups. The increase of TAT activity was about 214% (threefold) and 368% (fivefold) after 12 and 24 h of the CPF administration, respectively (Fig. 2A).

3.5. G6Pase activity

The Student's *t*-test of G6Pase activity demonstrated that CPF increased the enzyme activity in livers of rats after 12 ($p = 0.0059$) and 24 h ($p < 0.0001$) of exposure when compared to the corresponding control groups. The increase in G6Pase activity was about 25% and 31% following 12 and 24 h of the CPF administration, respectively (Fig. 2B).

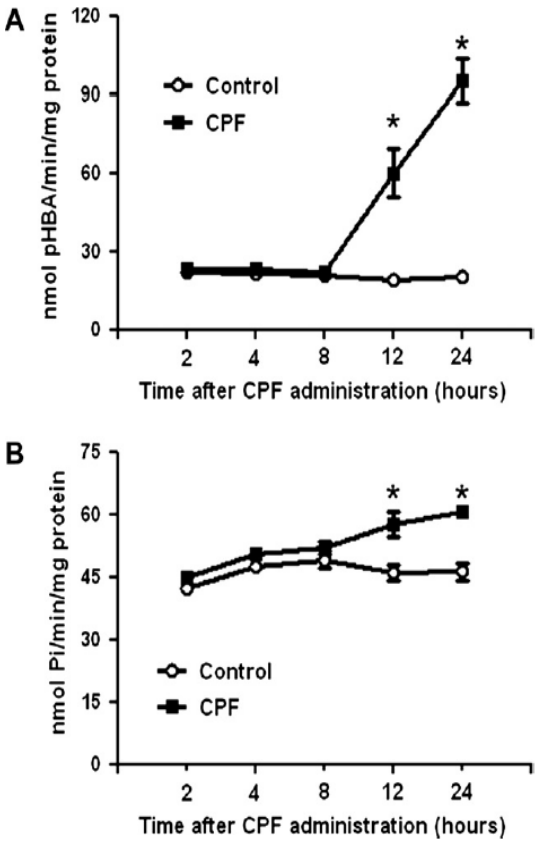

Fig. 2. Effect of CPF acute administration (50 mg kg^{-1}; s.c.) on TAT (A) and G6Pase (B) activities in livers in rats. Data are reported as the mean (s) ± S.E.M. of eight animals per group and expressed as nmol pHBA min^{-1} mg^{-1} protein and nmol Pi min^{-1} mg^{-1} protein, respectively. (*) Denotes $p < 0.05$ as compared to the control group of the corresponding exposure time (unpaired Student's *t*-test).

3.6. Corticosterone levels

The Student's *t*-test of corticosterone levels data demonstrated that CPF increased the hormone levels in plasma of rats after 12 ($p = 0.0105$) and 24 ($p = 0.0173$) h of exposure when compared to those of the corresponding control groups. In rats exposed to CPF for 12 and 24 h, corticosterone levels were about 20% and 24% higher than those of the corresponding control rats, respectively (Fig. 3).

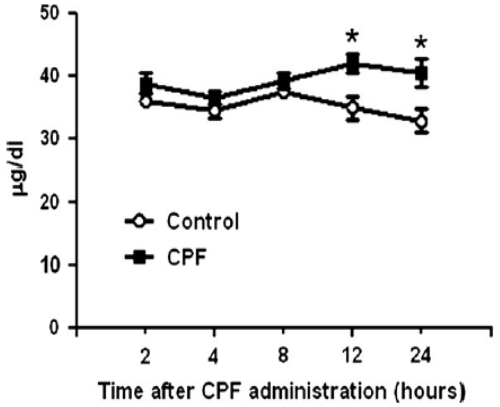

Fig. 3. Effect of CPF acute administration (50 mg kg^{-1}; s.c.) on plasma corticosterone levels in rats. Data are reported as the mean (s) ± S.E.M. of eight animals per group and expressed as µg dL^{-1}. (*) Denotes $p < 0.05$ as compared to the control group of the corresponding exposure time (unpaired Student's *t*-test).

3.7. AChE activity

The Student's *t*-test of AChE activity data revealed that CPF inhibited the enzyme activity in brains of rats after all analyzed time points when compared to the corresponding control groups. The inhibition of AChE activity was about 13% ($p = 0.0316$), 17% ($p = 0.0011$), 44% ($p < 0.0001$), 61% ($p < 0.0001$) and 86% ($p < 0.0001$) after 2, 4, 8, 12 and 24 h of the CPF administration, respectively (Fig. 4A).

3.8. Correlation analysis between corticosterone levels and gluconeogenic enzyme activities

The Pearson's correlation analysis revealed a positive correlation between plasma corticosterone levels and hepatic TAT and G6Pase activities in the CPF-exposed rats ($r = +0.47$; $p = 0.002$ and $r = +0.36$; $p = 0.0019$, respectively; Fig. 5A and B).

3.9. PON1 activity

The Student's *t*-test of PON1 activity data showed that CPF inhibited the enzyme activity in plasma of rats after 12 ($p = 0.0002$) and 24 h ($p < 0.0001$) of exposure when compared to those of the corresponding control groups. The reduction of PON1 activity was about 19% and 49% after 12 and 24 h of the CPF administration, respectively (Fig. 4B).

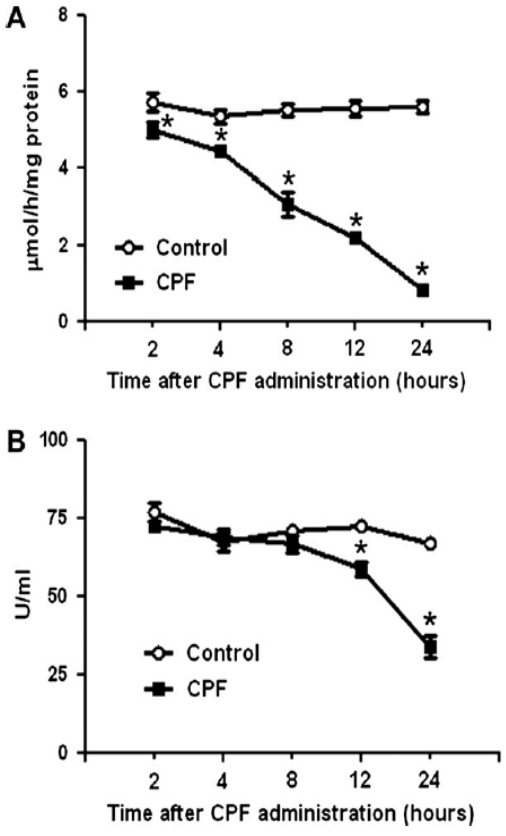

Fig. 4. Effect of CPF acute administration (50 mg kg^{-1}; s.c.) on cerebral AChE (A) and plasma PON1 (B) activities in rats. Data are reported as mean ± S.E.M. of eight animals per group and expressed as µmol h^{-1} mg^{-1} protein and U mL^{-1}, respectively. (*) Denotes $p < 0.05$ as compared to the control group of the corresponding exposure time (unpaired Student's *t*-test).

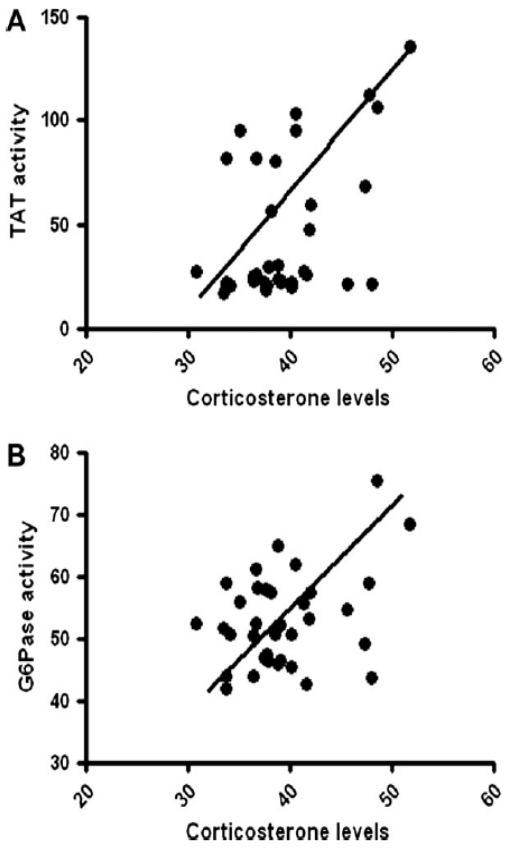

Fig. 5. Correlations between corticosterone levels and gluconeogenic enzyme activities in CPF-exposed rats. (A) Positive correlation between corticosterone levels and TAT activity; (B) positive correlation between corticosterone levels and G6Pase activity. Data are individual values from all animals over all time periods.

3.10. Lipid levels

The Student's *t*-test of total TC levels did not reveal alterations (Table 1).

The Student's *t*-test showed a decrease of HDL levels in plasma of rats exposed to CPF for 8–24 h when compared to the corresponding control groups. The decrease of HDL levels was about 21% ($p = 0.0002$), 16% ($p = 0.0058$) and 25% ($p = 0.0003$) following 8, 12 and 24 h of the CPF administration, respectively (Table 1).

The Student's *t*-test revealed that CPF induced an increase of LDL levels in plasma of rats after 8–24 h of exposure when compared to those of the corresponding control groups. The increase of LDL levels was about 45% ($p = 0.008$), 57% ($p = 0.0018$) and 74% ($p < 0.0001$) following 8, 12 and 24 h of the CPF administration, respectively (Table 1).

The Student's *t*-test showed that CPF increased TG levels in plasma of rats after all analyzed time points when compared to those of the corresponding control groups. Plasma TG levels measured at 2, 4, 8, 12 and 24 h following the CPF administration were about 51% ($p = 0.001$), 61% ($p = 0.0082$), 83% ($p = 0.0005$), 44% ($p = 0.0008$) and 91% ($p = 0.0005$) higher than those of the corresponding control rats, respectively (Table 1).

The Student's *t*-test showed an increase of cardiovascular factors, TC/HDL and TG/HDL and the atherogenic index (AI) of rats exposed to CPF when compared to the corresponding control groups. The increase in TC/HDL, TG/HDL and AI was observed after 8–24, 2–24 and 8–24 h of the CPF administration, respectively (Table 2).

4. Discussion

In the current study, we reported the hyperglycemic and hyperlipidemic effects of CPF after a single acute administration in rats. The CPF exposure caused an increase in plasma glucose and hepatic glycogen levels and an activation of the HPA axis, as demonstrated by an increase in corticosterone levels in plasma of rats. Moreover, we demonstrated an increase in the hepatic activities of TAT and G6Pase, enzymes involved in the gluconeogenesis pathway, an inhibition of cerebral AChE activity and a reduction of plasma PON1 activity. A significant correlation between corticosterone levels and the activities of TAT and G6Pase was also demonstrated. Regarding the lipid status, the CPF exposure caused an increase in plasma TG and LDL levels and a decrease in HDL levels associated with an increase of cardiovascular risk factors and the atherogenic index in rats.

Evidence has been found to suggest hyperglycemia as a characteristic outcome of OPs poisoning (Rahimi and Abdollahi, 2007). Hyperglycemia in experimental animals following acute exposure to OPs appears to be rapid in onset and transient in nature (Joshi and Rajini, 2012; Lasram et al., 2008). In accordance with these studies, we observed hyperglycemia after a single CPF administration in rats. However, in disagreement with these findings, we did not found a transient hyperglycemia, but a gradual increase of glucose levels until the time point, with the onset at 8 h and the maximum increase at 24 h after the CPF administration. It is important to consider that the route of OPs administration used in the cited studies was different from that of used in the present study. In fact, in the studies above cited OP was administered by the oral route whereas, in the present study, CPF was administered by the subcutaneous route. One possible explanation for the discrepancy found in these studies is that the absorption of CPF by the subcutaneous route was probably slower and; as a consequence, we observed a later and not transient hyperglycemia.

Table 1
Effect of CPF acute administration on lipid status of rats.

Time (h)	Group	TC	HDL	LDL	TG
2	Control	73.17 ± 2.98	39.00 ± 0.96	25.40 ± 2.96	43.83 ± 2.16
	CPF	78.50 ± 4.25	40.50 ± 1.76	29.43 ± 2.40	66.33 ± 4.99*
4	Control	74.33 ± 2.43	39.00 ± 0.68	28.67 ± 3.09	40.00 ± 1.75
	CPF	80.00 ± 3.26	40.00 ± 1.94	28.93 ± 2.63	64.33 ± 7.71*
8	Control	75.17 ± 3.53	41.17 ± 1.39	23.47 ± 2.43	36.00 ± 2.88
	CPF	77.83 ± 3.09	32.33 ± 1.13*	34.03 ± 2.40*	66.00 ± 5.97*
12	Control	76.00 ± 5.48	40.00 ± 1.19	23.63 ± 2.47	43.50 ± 2.84
	CPF	82.00 ± 3.67	33.50 ± 1.60*	37.20 ± 2.50*	62.83 ± 3.58*
24	Control	69.50 ± 3.11	39.00 ± 1.18	18.20 ± 1.29	42.83 ± 3.86
	CPF	72.67 ± 3.21	29.33 ± 1.67*	31.65 ± 1.43*	81.89 ± 7.83*

Data are reported as the mean (s) ± S.E.M. of eight animals per group.
* $p < 0.05$ as compared to the control group of the corresponding exposure time (unpaired Student's *t*-test). TC, HDL, LDL and TG levels were expressed as mg dL^{-1}.

Table 2
Effect of CPF acute administration on cardiovascular risk factors and atherogenic index of rats.

Time (h)	Group	TC/HDL	TG/HDL	AI [(TC–HDL)/HDL]
2	Control	1.88 ± 0.08	1.12 ± 0.05	0.88 ± 0.08
	CPF	1.94 ± 0.09	1.66 ± 0.14*	0.94 ± 0.09
4	Control	1.91 ± 0.06	1.03 ± 0.05	0.91 ± 0.06
	CPF	2.05 ± 0.16	1.64 ± 0.20*	1.05 ± 0.16
8	Control	1.84 ± 0.10	0.88 ± 0.08	0.84 ± 0.10
	CPF	2.41 ± 0.04*	2.06 ± 0.20*	1.41 ± 0.04*
12	Control	1.90 ± 0.12	1.09 ± 0.06	0.90 ± 0.12
	CPF	2.46 ± 0.09*	1.89 ± 0.11*	1.46 ± 0.09*
24	Control	1.78 ± 0.07	1.10 ± 0.09	0.78 ± 0.07
	CPF	2.52 ± 0.16*	2.93 ± 0.4*	1.52 ± 0.16*

Data are reported as the mean (s) ± S.E.M. of eight animals per group.
* $p < 0.05$ as compared to the control group of the corresponding exposure time (unpaired Student's *t*-test).

The mechanisms involved in the hyperglycemia induced by OPs have been under investigation in the recent years. One of the mechanisms proposed for the OPs-induced hyperglycemia is the activation of the HPA axis that regulates the process of synthesis and secretion of glucocorticoids by the adrenal cortex (Rahimi and Abdollahi, 2007). In the stress response, hypothalamus releases the corticotropin-releasing hormone (CRH) that stimulates pituitary to release the adrenocorticotropic hormone (ACTH). ACTH subsequently stimulates the secretion of glucocorticoids from adrenal gland (Beishuizen and Thijs, 2003). Glucocorticoid hormones (mainly cortisol in man and corticosterone in rodents) increase blood glucose by induction of gluconeogenesis pathway (Khani and Tayek, 2001). OPs have been shown to elicit activation of HPA leading to an increase in plasma corticosterone levels (Joshi and Rajini, 2009, 2012). Accordingly, in the current study we showed an increase in plasma corticosterone levels associated with the increase of TAT and G6Pase activities after 12 and 24 h of the CPF administration. In addition to these effects, a positive correlation between corticosterone levels and TAT and G6Pase activities in the CPF-exposed rats was demonstrated. Thus, the animals that had the highest corticosterone levels had the highest TAT and G6Pase activities. Taken these results collectively one can affirm that an increase in the gluconeogenesis pathway, probably elicited by hypercorticosteronemia, may be the mechanism behind CPF-induced hyperglycemia in rats. Furthermore, the stimulation of HPA axis, found in this study, can be explained by the inhibition of cerebral AChE. In fact, studies have found evidence indicating that ACh stimulates the release of CRH from hypothalamus in experimental animals (Bugajski et al., 2001, 2007).

The activation of the glycogenolysis pathway (Abdollahi et al., 2004) has been reported as a possible mechanism involved in hyperglycemia caused by OPs. However, there are different reports regarding the effects of OPs exposure in the hepatic glycogen levels. Acute exposure to malathion showed a relationship between increased plasma glucose levels and decreased hepatic glycogen levels, indicating that the glycogenolysis pathway plays an important role in the hyperglycemia caused by this insecticide (Lasram et al., 2008). Conversely, exposure to acephate increased glucose levels and did not alter glycogen levels, indicating that the glycogenolysis pathway is not involved in its hyperglycemic effect (Joshi and Rajini, 2009). In the present study, we demonstrated that the hyperglycemia caused by CPF was not associated with the activation of the glycogenolysis pathway and, in disagreement with these reports; we found an increase in glycogen levels after 12 and 24 h of the CPF administration. It is important to highlight that these above reported studies investigated the effects of other OPs and not CPF in the glycogenolysis pathway. Therefore, one can suggest that the increase in TAT and G6Pase activities, and not the activation of the glycogenolysis pathway, is related to the hyperglycemia caused by CPF.

TAT is a gluconeogenic enzyme that catalyzes the degradation of tyrosine to form p-hydroxyphenylpyruvate (Dundjerski et al., 2003). G6Pase in turn catalyzes the final reaction of gluconeogenesis, the dephosphorylation of glucose 6-phosphate (G6P) to yield glucose. On the other hand, to initiate the hepatic glycogen synthesis, G6P is converted into glucose 1-phosphate (G1P) by the action of phosphoglucomutase. The product of this reaction is converted into UDP-glucose in a key step of glycogen biosynthesis (Radziuk and Pye, 2001). In the present study, the increase in TAT (about threefold and fivefold after 12 and 24 h of CPF administration, respectively) was not proportional to the increase of G6Pase activity (about 25% and 31%, respectively). However, at the same CPF exposure time points we found a marked increase of glycogen levels (about 82% and six fold, respectively). These results suggest that the increase of TAT activity, resulting from the HPA axis activation, produces more p-hydroxyphenylpyruvate that in turn is converted into G6P by the gluconeogenesis pathway. Only a small portion of G6P formed is converted into glucose by the action of G6Pase increasing plasma glucose levels. The remaining G6P probably is converted into G1P by the action of phosphoglucomutase, serving as a substrate for the glycogen biosynthesis, which explains the great increase of glycogen levels found in the livers of rats exposed to CPF.

In the present study, we also demonstrated the hyperlipidemic effect of CPF after an acute administration in rats. An increase in triglycerides and LDL levels and a decrease in HDL levels were found in plasma of CPF-exposed rats. These alterations were associated with the increase of cardiovascular risk factors and the atherogenic index. Accordingly, Lasram et al. (2009) demonstrated that a single malathion administration in rats caused hyperlipidemia and an increase of cardiovascular risk factors and the atherogenic index. Ibrahim and El-Gamal (2003) also reported that diazinon increased plasma triglyceride levels which were attributed to an inhibition of the lipase activity of both the hepatic triglycerides and plasma lipoproteins. Underlying mechanisms of CPF-induced hyperlipidemia are yet to be elucidated and at the moment, it is difficult to establish whether there is a relationship between hyperlipidemic and hyperglycemic effects of CPF. However, one can speculate that if these alterations remain over time, the CPF exposure may represent a risk factor for the development of cardiovascular diseases.

PON1 is a HDL-associated plasma enzyme that plays an important role in the toxicity of some OPs by hydrolyzing their toxic oxon metabolites (Furlong et al., 2010). In fact, low PON1 activity seems to be associated with higher pesticide sensitivity (Lacasaña et al., 2010). In addition to protecting against exposure to some OPs, PON1 is believed to have a protective role in the atherosclerotic process by both contributing to HDL's protective effect against atherosclerosis and preventing the LDL oxidation (Aviram and Rosenblat, 2005). In the present study, we showed a reduction of PON1 activity that may be related to the reduction of CPF-oxon detoxification and an increase in the sensitivity of animals to the toxic effects caused by CPF. The reduction of PON1 activity may not be related to the inhibition by CPF since A-esterases are not considered to be enzymes inhibited following exposure to OPs (Costa et al., 2002). However, whereas PON1 is part of the HDL complex, one could explain the reduction of the enzyme activity by the reduced HDL levels found in plasma of CPF-exposed rats. Because PON1 protects against the development of vascular diseases, the reduction of this enzyme activity, if sustained, reinforces the hypothesis that CPF exposure may represent a risk factor to the development of cardiovascular diseases.

5. Conclusion

In conclusion, the present study demonstrated that a single acute administration of CPF caused hyperglycemic and hyperlipidemic effects in rats. The activation of the gluconeogenesis pathway, probably elicited by hypercorticosteronemia, was proven to be involved in the hyperglycemic effect of CPF in rats. The mechanisms involved in the CPF-induced hyperlipidemia are yet to be elucidated; however, if these alterations remain, the CPF exposure may represent a risk factor for the development of cardiovascular diseases.

Acknowledgments

The authors thank to CAPES, CNPq, FAPERGS/CNPq (PRONEX) research Grant # 10/0005-1 and FAPERGS research Grant # 10/0711-6. The granting agency had no role in the study design, analysis or interpretation of data, writing the paper or in submitting the paper for publication.

References

Abdollahi, M., Donyavi, M., Pournourmohammadi, S., Saadat, M., 2004. Hyperglycemia associated with increased hepatic glycogen phosphorylase and phosphoenolpyruvate carboxykinase in rats following sub chronic exposure to malathion. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 137, 247–343.

Aviram, M., Rosenblat, M., 2005. Paraoxonases and cardiovascular diseases: pharmacological and nutritional influences. Curr. Opin. Lipidol. 16, 393–399.

Ayub, A., Mackness, M.I., Arrol, S., Mackness, B., Patel, J., Durrington, P.M., 1999. Serum paraoxonase after myocardial infarction. Arterioscler. Thromb. Vasc. Biol. 19, 330–335.

Beishuizen, A., Thijs, L.G., 2003. Endotoxin and the hypothalamo–pituitary–adrenal (HPA) axis. J. Endotoxin Res. 9, 3–24.

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

Bugajski, J., Gadek-Michalska, A., Bugajski, A.J., 2001. A single corticosterone pretreatment inhibits the hypothalamic–pituitary–adrenal responses to adrenergic and cholinergic stimulation. J. Physiol. Pharmacol. 52, 313–324.

Bugajski, A.J., Zurowski, D., Thor, P., Gadek-Michalska, A., 2007. Effect of subdiaphragmatic vagotomy and cholinergic agents in the hypothalamic–pituitary–adrenal axis activity. J. Physiol. Pharmacol. 58, 335–347.

Busby-Hjerpe, A.L., Campbell, J.A., Smith, J.N., Lee, S., Poet, T.S., Barr, D.B., Timchalk, C., 2010. Comparative pharmacokinetics of chlorpyrifos versus its major metabolites following oral administration in the rat. Toxicology 268, 55–63.

Chen, X.P., Wang, X., Dong, J.Y., 2011. Different reaction patterns of dopamine content to prenatal exposure to chlorpyrifos in different periods. J. Appl. Toxicol. 31, 355–359.

Costa, L.G., Li, W.F., Richter, R.J., Shih, D.M., Lusis, A.J., 2002. PON1 and organophosphate toxicity. In: Costa, L.G., Furlong, C.E. (Eds.), Paraoxonase (PON1) in Health and Disease: Basic and Clinical Aspects. MA' Kluwer Academic Publishers, Norwell, pp. 165–183.

Çetin, E., Kanbur, M., Silici, S., Eraslan, G., 2010. Propetamphos-induced changes in haematological and biochemical parameters of female rats: protective role of propolis. Food Chem. Toxicol. 48, 1806–11810.

Diamondstone, T.I., 1966. Assay of tyrosine transaminase activity by conversion of *p*-hydroxyphenylpyruvate to *p*-hydroxybenzaldehyde. Anal. Biochem. 16, 395–401.

Dobrat, W., Martijn, A. (Eds.), 1998. CIPAC Handbook Volume H: Analysis of Technical and Formulated Pesticides. Black Bear Press, King's Hedges Road, Cambridge, UK. pp. 359.

Dundjerski, J., Predic, J., Cvoro, A., Matic, G., 2003. Rat liver tyrosine aminotransferase activity and induction by dexamethasone upon cadmium intoxication. Arch. Biol. Sci. Belgrade 55, 3–7.

Ellman, G.L., Courtney, K.D., Andres, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

Fiske, C.H., Subbarow, Y.J., 1925. The colorimetric determination of phosphorus. Biol. Chem. 66, 375–381.

Furlong, C.E., Suzuki, S.M., Stevens, R.C., Marsillach, J., Richter, R.J., Jarvik, G.P., Checkoway, H., Samii, A., Costa, L.G., Griffith, A., Roberts, J.W., Yearout, D., Zabetian, C.P., 2010. Human PON1, a biomarker of risk of disease and exposure. Chem. Biol. Interact. 187, 355–361.

Galloway, T., Handy, R., 2003. Immunotoxicities of organophosphorus pesticides. Ecotoxicology 12, 345–363.

Ibrahim, N.A., El-Gamal, B.A., 2003. Effect of diazinon, an organophosphate insecticide, on plasma lipid constituents in experimental animals. J. Biochem. Mol. Biol. 36, 499–504.

Joshi, A.K.R., Rajini, P.S., 2009. Reversible hyperglycemia in rats following acute exposure to acephate, an organophosphorus insecticide: role of gluconeogenesis. Toxicology 257, 40–45.

Joshi, A.K.R., Rajini, P.S., 2012. Hyperglycemic and stressogenic effects of monocrotophos in rats: evidence for the involvement of acetylcholinesterase inhibition. Exp. Toxicol. Pathol. 64, 115–120.

Kamath, V., Rajini, P.S., 2007. Altered glucose homeostasis and oxidative impairment in pancreas of rat subjected to dimethoate intoxication. Toxicology 231, 137–146.

Kamath, V., Joshi, A.K.R., Rajini, P.S., 2008. Dimethoate induced biochemical perturbations in rat pancreas and its attenuation by cashew nut skin extract. Pest. Biochem. Physiol. 90, 58–65.

Khani, S., Tayek, J.A., 2001. Cortisol increases gluconeogenesis in humans: its role in the metabolic syndrome. Clin. Sci. (Lond.) 101, 739–747.

Kousba, A.A., Sultatos, L.G., Poet, T.S., Timchalk, C., 2004. Comparison of chlorpyrifos-oxon and paraoxon acetylcholinesterase inhibition dynamics: potential role of a peripheral binding site. Toxicol. Sci. 80, 239–248.

Krisman, C.R., 1962. A method for the colorimetric estimation of glycogen with iodine. Anal. Biochem. 4, 17–23.

Kwong, T.C., 2002. Organophosphate pesticides: biochemistry and clinical toxicology. Ther. Drug Monit. 24, 144–149.

Lacasaña, M., López-Flores, I., Rodríguez-Barranco, M., Aguilar-Garduño, C., Blanco-Muñoz, J., Pérez-Méndez, O., Gamboa, R., Gonzalez-Alzaga, B., Bassol, S., Cebrian, M.E., 2010. Interaction between organophosphate pesticide exposure and PON1 activity. Toxicol. Appl. Pharmacol. 249, 16–24.

Lasram, M.M., Annabi, A.B., Rezg, R., Elj, N., Slimen, S., Kamoun, A., El-Fazaa, S., Gharbi, N., 2008. Effect of short-time malathion administration on glucose homeostasis in Wistar rat. Pestic. Biochem. Physiol. 92, 114–119.

Lasram, M.M., Annabi, A.B., Elj, N.E., Selmi, S., Kamoun, A., El-Fazaa, S., Gharbi, N., 2009. Metabolic disorders of acute exposure to malathion in adult Wistar rats. J. Hazard. Mater. 163, 1052–1055.

Mehta, A., Verma, R.S., Srivastava, N., 2008. Chlorpyrifos-induced DNA damage in rat liver and brain. Environ. Mol. Mutagen. 49, 426–433.

Radziuk, J., Pye, S., 2001. Hepatic glucose uptake, gluconeogenesis and the regulation of glycogen synthesis. Diabetes Metab. Res. Rev. 17, 250–272.

Rahimi, R., Abdollahi, M., 2007. A review on mechanisms involved in hyperglycemia induced by organophosphorus insecticides. Pest. Biochem. Physiol. 88, 115–121.

Reaven, G.M., 2003. Importance of identifying the overweight patient who will benefit the most by losing weight. Ann. Intern. Med. 138, 420–423.

Ricketts, T.R., 1963. An improved micromethod for the determination of glucose-6 phosphatase activity. Clin. Chim. Acta 8, 160–162.

Rusyniak, D.E., Nanagas, K.A., 2004. Organophosphate poisoning. Semin. Neurol. 24, 197–204.

Soltaninejad, K., Abdollahi, M., 2009. Current opinion on the science of organophosphate pesticides and toxic stress: a systematic review. Med. Sci. Monit. 15, 75–90.

Tian, Y., Ishikawa, H., Yamaguchi, T., Yamauchi, T., Yokoyama, K., 2005. Teratogenicity and developmental toxicity of chlorpyrifos maternal exposure during organogenesis in mice. Reprod. Toxicol. 20, 267–271.

Verma, R.S., Srivastava, N., 2003. Effect of chlorpyrifos on thiobarbituric acid reactive substances, scavenging enzymes and glutathione in rat tissues. Indian J. Biochem. Biophys. 40, 423–428.

Wu, H., Zhang, R., Liu, J., Guo, Y., Ma, E., 2011. Effects of malathion and chlorpyrifos on acetylcholinesterase and antioxidant defense system in Oxya chinensis (Thunberg) (Orthoptera: Acrididae). Chemosphere 83, 599–604.

Zenker, N., Bernstein, D.E., 1958. The estimation of small amounts of corticosterone in rat plasma. J. Biol. Chem. 231, 695–701.

4 DISCUSSÃO

A aplicação intensiva de agrotóxicos tem causado sérias conseqüências ao meio ambiente e à saúde dos seres humanos, principalmente aos trabalhadores rurais. A intoxicação por agrotóxicos é um problema de saúde pública grave, principalmente nos países em desenvolvimento e nos emergentes. As intoxicações agudas merecem especial destaque uma vez que a falta de instrução e de cuidado na aplicação desses produtos é muito freqüente, o que atribui aos agrotóxicos um grau de destaque como poderosos agentes de contaminação humana (Domingues, 2004). Entre os agrotóxicos relacionados com casos de intoxicação em humanos, destacam-se os OFs tais como o CPF e o AC (Panemangalore *et al*., 1999; Singh *et al*., 2011).

Dessa forma, no **artigo 1**, verificou-se o efeito da exposição aguda ao CPF em fígado de ratos. O presente trabalho demonstrou que a exposição ao CPF causou um dano hepático evidenciado pelo aumento da atividade das enzimas alanina aminotransferase (ALT), aspartato aminotransferase (AST) e lactato desidrogenase (LDH) no plasma dos ratos. O aumento da atividade dessas enzimas no plasma indica um aumento da permeabilidade dos hepatócitos, o que permite o extravasamento das mesmas para a corrente sangüínea (Gokcimen *et al*., 2007). O dano hepático causado pelo CPF esteve associado ao estresse oxidativo, confirmado pelo aumento da peroxidação lipídica e carbonilação de proteínas, bem como, pela diminuição das defesas antioxidantes enzimáticas (atividade da catalase (CAT), superóxido dismutase (SOD), glutationa peroxidase (GPx), glutationa S-transferase (GST)) e não-enzimáticas (níveis de tióis não-protéicos (SHNP)) no fígado dos animais expostos ao CPF. Estes resultados estão de acordo com outros trabalhos que mostraram o efeito hepatotóxico do CPF em ratos (Khan e Kour, 2007; Verma *et al*., 2007).

Embora o exato mecanismo pelo qual os OFs causam estresse oxidativo ainda não esteja completamente elucidado, acredita-se que o aumento da produção de espécies reativas de oxigênio (EROs) seja uma decorrência da metabolização dessas substâncias pelo citocromo P450 (Lukaszewicz-Hussain, 2010). As enzimas do citocromo P450 são monooxigenases que catalisam a adição de um átomo de oxigênio no substrato (organofosforado), reação na qual as EROs são geradas (Chambers *et al*., 2001). Além disso, outros mecanismos como a inibição dos complexos da cadeia de transporte de elétrons mitocondrial e a indução de hiperglicemia

também parecem estar envolvidos no estresse oxidativo induzido pelos OFs (Lukaszewicz-Hussain, 2010).

No **artigo 1** avaliou-se também o efeito da exposição aguda ao CPF em parâmetros hematológicos de ratos. Os resultados demonstraram que o CPF causou a diminuição dos níveis de leucócitos totais e de linfócitos e o aumento dos níveis de monócitos, neutrófilos, hemoglobina e hematócrito. A acentuada diminuição dos níveis de leucócitos totais, um marcador de defesa celular, pode ser explicada pela diminuição da produção dessas células ou pela migração das mesmas para os sítios de dano celular causado pelo CPF. Já o aumento dos níveis de neutrófilos e monócitos, primeira e segunda linhas de defesa do organismo contra o dano oxidativo (Kobayashi *et al*., 2003), pode ter ocorrido como conseqüência da injúria hepática causada pelo CPF. O discreto aumento dos níveis de hemoglobina e hematócrito nos animais expostos ao CPF não foi considerado importante uma vez que esses níveis ainda permaneceram dentro da faixa dos valores de referência para ratos. Outros autores demonstraram efeitos semelhantes após a exposição à OFs em ratos (Goel *et al*., 2006; Çetin *et al*., 2010).

No **artigo 2** foram investigados os distúrbios metabólicos induzidos pela exposição aguda ao AC em ratos. Os resultados deste trabalho demonstraram que o AC causou o aumento dos níveis de glicose e corticosterona plasmática, o aumento da atividade das enzimas hepáticas TAT e G6Pase e a inibição da AChE cerebral. De acordo com Joshi e Rajini (2009), esses resultados indicam que a ativação do HPA é o mecanismo envolvido na hiperglicemia induzida pelo AC. Observou-se também que a exposição ao AC causou o aumento dos níveis de TG plasmáticos e do fator de risco cardiovascular TG/HDL.

Considerando o número crescente de intoxicações e o fato de que os seres humanos estão cada vez mais expostos aos OFs, torna-se de fundamental importância a busca por novos compostos capazes de diminuir os efeitos tóxicos causados pelos OFs. Nesse contexto, destaca-se o $(PhSe)_2$, um composto orgânico de selênio bastante estudado por nosso grupo de pesquisa e que apresenta diversas propriedades farmacológicas já descritas. Dentre elas, as de maior interesse para este trabalho são as atividades antioxidante (Prigol *et al*., 2009a), hepatoprotetora (Borges *et al*., 2008), anti-hiperglicêmica (Barbosa *et al*., 2006) e anti-hiperlipidêmica (da Rocha *et al*., 2009).

Dessa forma, verificou-se que o pré-tratamento com $(PhSe)_2$ foi efetivo em proteger contra o estresse oxidativo hepático causado pela exposição ao CPF no **artigo 1**, demonstrando assim o seu efeito antioxidante. Além disso, o pré-tratamento com $(PhSe)_2$ aumentou *per se* os níveis de SHNP e a atividade da GST. Outros trabalhos do nosso grupo de

pesquisa demonstraram que o $(PhSe)_2$ aumentou *per se* os níveis de SHNP e a atividade da GST (Barbosa *et al*., 2006; Borges *et al*., 2008; Luchese *et al*., 2009), o que indica o envolvimento do sistema da glutationa no efeito antioxidante do $(PhSe)_2$. Verificou-se também que o $(PhSe)_2$ atenuou a diminuição dos níveis de leucócitos totais e o aumento dos níveis de monócitos decorrentes da exposição ao CPF. Esses resultados estão de acordo com outros trabalhos que demonstraram o efeito protetor do $(PhSe)_2$ contra as alterações hematológicas induzidas por mercúrio (Brandão *et al*., 2008; 2009).

O mecanismo envolvido no efeito antioxidante do $(PhSe)_2$ e já descrito na literatura envolve a sua redução mediada por grupos tióis com a conseqüente geração de intermediários selenol (fenilselenol). Esses intermediários fenilselenol são capazes de reagir com peróxidos lipídicos formando um álcool inerte, interrompendo dessa forma a fase de propagação da peroxidação lipídica (Nogueira e Rocha, 2010) (Figura 1). Além disso, foi demonstrado que o $(PhSe)_2$ apresenta atividade mimética das enzimas GST e dehidroascorbato redutase, atividade esta que é dependente da concentração de glutationa presente no meio (Luchese e Nogueira, 2010).

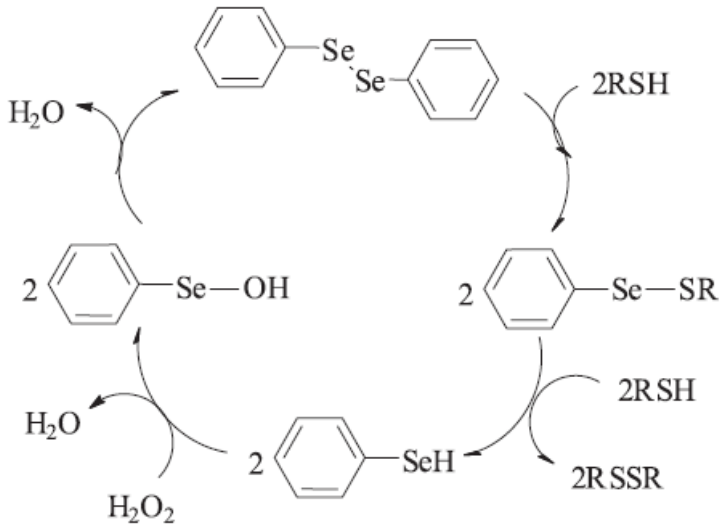

Figura 1 – Mecanismo proposto para o efeito antioxidante do disseleneto de difenila [$(PhSe)_2$].
Fonte: Nogueira e Rocha, 2010.

No **artigo 2** demonstrou-se o efeito protetor do $(PhSe)_2$ contra os distúrbios metabólicos induzidos pelo AC. Os resultados mostraram que o $(PhSe)_2$ atenuou o aumento dos níveis de glicose decorrentes da exposição ao AC, demonstrando assim o seu efeito anti-

hiperglicêmico. Trabalhos do nosso grupo de pesquisa demonstraram que o $(PhSe)_2$ diminuiu os níveis de glicose em ratos diabéticos (Barbosa *et al*., 2008; Kade *et al*., 2009).

Com relação aos mecanismos envolvidos no efeito anti-hiperglicêmico do $(PhSe)_2$ no presente trabalho, pôde-se descartar o envolvimento do eixo HPA, uma vez que o $(PhSe)_2$ não foi capaz de proteger contra a inibição da AChE cerebral e o aumento dos níveis de corticosterona plasmática. No entanto, verificou-se que o $(PhSe)_2$ de alguma forma modula a via da gliconeogênese, uma vez que houve uma proteção contra o aumento da atividade das enzimas TAT e G6Pase no fígado dos animais expostos ao AC. Além disso, verificou-se que o $(PhSe)_2$ em ambas as doses protegeu de maneira similar contra o aumento dos níveis de glicose, entretanto, a dose de 30 mg/kg foi mais eficaz em proteger contra o aumento da atividade das enzimas TAT e G6Pase do que a dose de 10 mg/kg. Esses resultados indicam que provavelmente outros mecanismos, além da modulação da via da gliconeogênese, estejam envolvidos no efeito anti-hiperglicêmico do $(PhSe)_2$. No entanto, esses mecanismos ainda necessitam ser elucidados.

Além do efeito anti-hiperglicêmico, no **artigo 2** verificou-se também o efeito anti-hiperlipidêmico do $(PhSe)_2$, evidenciado pela proteção contra o aumento dos níveis de TG e do fator de risco cardiovascular (TG/HDL) decorrente da exposição ao AC. Estudos do nosso grupo de pesquisa demonstraram que o $(PhSe)_2$ possui efeito anti-hiperlipidêmico em outros modelos experimentais (da Rocha *et al*., 2009; 2011). No entanto, os mecanismos através dos quais o $(PhSe)_2$ exerce esse efeito anti-hiperlipidêmico ainda não foram elucidados.

No **artigo 3** foram avaliados os efeitos hiperglicêmico e hiperlipidêmico do CPF em ratos, bem como os mecanismos envolvidos na hiperglicemia induzida por esse inseticida. Os resultados obtidos nesse trabalho demonstraram que o CPF, após uma única administração, causou o aumento dos níveis de glicose a partir do tempo de 8 horas e esse aumento permaneceu até 24 horas. O aumento dos níveis de glicose tem sido demonstrado como uma das conseqüências da exposição a diferentes OFs em diversos modelos experimentais (Kamath e Rajini, 2006; Rezg *et al*., 2007). A hiperglicemia causada pelos OFs geralmente inicia rapidamente e é transitória (Lasram *et al*., 2008; Joshi e Rajini, 2009). No entanto, em desacordo com os trabalhos anteriormente citados, no presente trabalho não se observou uma hiperglicemia transitória, e sim, um aumento gradual dos níveis de glicose até o tempo avaliado (24 horas). Essa diferença de efeito pode ser atribuída ao uso de um OF diferente e à administração do mesmo por uma via diferente.

A hiperglicemia induzida pelo CPF demonstrada no **artigo 3** pode estar relacionada ao estresse oxidativo hepático causado por esse inseticida e demonstrado no **artigo 1**. De fato,

para esses dois trabalhos foram empregadas as mesmas doses e a mesma via de administração do CPF. Embora os níveis de glicose não tenham sido determinados no **artigo 1**, provavelmente os animais expostos ao CPF permaneceram com hiperglicemia até o final do tratamento. Isso pode ser sugerido considerando-se que no **artigo 3** não foi observada a reversão da hiperglicemia até o tempo de 24 horas e que após esse período os animais do **artigo 1** receberam uma segunda administração de CPF e foram mortos 24 horas após a mesma. A hiperglicemia leva ao aumento da glicação não-enzimática de proteínas e conseqüentemente à formação dos produtos finais de glicação avançada (AGEs). Os AGEs ativam receptores específicos de membrana (RAGEs) e induzem a produção intracelular de EROs levando ao desenvolvimento do estresse oxidativo (Ceriello, 1997; Gillery, 2006). Dessa forma, provavelmente a hiperglicemia está envolvida na indução de estresse oxidativo hepático pelo CPF.

Os mecanismos envolvidos na hiperglicemia induzida pelo CPF também foram investigados no **artigo 3.** Verificou-se um aumento inesperado dos níveis de glicogênio hepático em 12-24 horas após a exposição ao CPF, o que descarta o envolvimento da glicogenólise na hiperglicemia induzida por esse inseticida. No entanto, os animais expostos ao CPF após 12-24 horas apresentaram um aumento dos níveis de corticosterona plasmática bem como um aumento da atividade das enzimas gliconeogênicas TAT e G6Pase. Demonstrou-se também uma correlação positiva entre os níveis de corticosterona e a atividade das enzimas TAT e G6Pase. Esses resultados sugerem que a hiperglicemia induzida pelo CPF é resultado da estimulação do eixo HPA e da conseqüente ativação da via da gliconeogênese. Além disso, a estimulação do eixo HPA pode ter ocorrido devido à inibição da AChE cerebral. O excesso de ACh decorrente da inibição da AChE pode ter estimulado o hipotálamo a liberar o CRH que conseqüentemente estimula todo o eixo HPA. Alguns autores também demonstraram o envolvimento da ativação do eixo HPA na hiperglicemia induzida por outros OFs (Joshi e Rajini 2009; 2012).

O aumento inesperado dos níveis de glicogênio nos animais expostos ao CPF pode ser explicado considerando-se o aumento não-proporcional da atividade das enzimas TAT e G6Pase. O aumento da atividade da TAT (cerca de 3 e 5 vezes após 12 e 24 horas da exposição ao CPF, respectivamente) forneceu um aumento dos níveis de glicose-6-fosfato. Como o aumento da atividade da G6Pase (cerca de 25 e 31%, após 12 e 24 horas da exposição ao CPF, respectivamente) não foi proporcional ao aumento da atividade da TAT, sugere-se que apenas uma pequena porção da glicose-6-fosfato formada tenha sido convertida em glicose livre pela G6Pase. A glicose-6-fosfato remanescente provavelmente foi convertida em

glicose-1-fosfato pela ação da fosfoglicomutase, servindo assim como substrato para a glicogênese.

Além do efeito hiperglicêmico do CPF, investigou-se também o seu efeito hiperlipidêmico. Os resultados do **artigo 3** mostraram que os animais expostos ao CPF tiveram um aumento dos níveis de TG e colesterol LDL bem como uma diminuição dos níveis de colesterol HDL, o que caracteriza a hiperlipidemia induzida pelo CPF. Observou-se ainda um aumento dos fatores de risco cardiovascular e do índice aterogênico. Os resultados desse estudo estão de acordo com outros autores que demonstraram o efeito hiperlipidêmico de outros OFs (Lasram *et al*., 2009; Çetin *et al*., 2010). Nesse trabalho não foi possível demonstrar o mecanismo pelo qual o CPF causa hiperlipidemia e nem se existe uma relação entre o seu efeito hiperglicêmico e hiperlipidêmico. No entanto, se essas alterações se mantiverem após o tempo avaliado (24 horas), a exposição ao CPF pode representar um fator de risco para o desenvolvimento de doenças cardiovasculares.

Outra alteração demonstrada no **artigo 3** foi a diminuição da atividade da PON-1. A diminuição da atividade dessa enzima provavelmente diminuiu a detoxificação do CPF-oxon e aumentou a suscetibilidade dos animais aos efeitos tóxicos do CPF demonstrados nesse trabalho. Além disso, considerando-se que a PON-1 é uma enzima associada à HDL, a diminuição da atividade da PON-1 provavelmente não ocorreu devido a uma inibição direta pelo CPF, e sim devido à diminuição dos níveis de HDL demontrada nos animais expostos ao CPF.

A análise destes 3 trabalhos aqui descritos permite um maior entendimento sobre os efeitos tóxicos causados pelos OFs CPF e AC, bem como revela a importância dos efeitos farmacológicos do $(PhSe)_2$ para diminuir os efeitos tóxicos induzidos por essas substâncias. Os resultados demonstraram que a exposição ao CPF causa toxicidade hepática e hematológica, hiperglicemia e hiperlipidemia em ratos. Os mecanismos envolvidos na hiperglicemia induzida pelo CPF são a ativação do HPA e da via da gliconeogênese. A exposição ao AC também causou hiperglicemia e hiperlipidemia em ratos. O $(PhSe)_2$ protegeu contra a toxicidade hepática e hematológica induzida pelo CPF e contra os distúrbios metabólicos causados pelo AC. Considerando-se que a exposição aos OFs é cada vez mais freqüente e que é a causa de diversas doenças, os resultados deste trabalho são de grande importância, uma vez que o $(PhSe)_2$ pode representar uma alternativa para atenuar a toxicidade causada pelos OFs.

5 CONCLUSÕES

De acordo com os resultados obtidos, pode-se concluir que:

- O $(PhSe)_2$ protegeu contra o dano oxidativo hepático causado pela exposição aguda ao CPF em ratos.
- O $(PhSe)_2$ atenuou a diminuição dos níveis de leucócitos totais e o aumento dos níveis de monócitos decorrentes da exposição aguda ao CPF em ratos.
- O $(PhSe)_2$ aumentou *per se* os níveis de SHNP e a atividade da enzima GST, indicando que o seu efeito antioxidante envolve a interação com o sistema da glutationa.
- O $(PhSe)_2$ protegeu contra a hiperglicemia e hiperlipidemia induzidas pela exposição aguda ao AC em ratos. A modulação da via da gliconeogênese, está envolvida, pelo menos em parte, no efeito anti-hiperglicêmico do $(PhSe)_2$.
- O CPF causou hiperglicemia e hiperlipidemia após uma exposição aguda em ratos. A hiperglicemia provavelmente é um dos mecanismos envolvidos no estresse oxidativo hepático causado pelo CPF.
- A ativação do eixo HPA e a conseqüente ativação da via da gliconeogênese estão envolvidas no efeito hiperglicêmico do CPF.

6 PERSPECTIVAS

Considerando os resultados obtidos nessa tese, as perpectivas para trabalhos posteriores são:

- Determinar os efeitos do $(PhSe)_2$ na hiperglicemia e hiperlipidemia induzida por CPF.
- Investigar os possíveis mecanismos envolvidos na ação farmacológica do $(PhSe)_2$ contra a toxicidade causada por CPF e AC.
- Avaliar se a co-administração de $(PhSe)_2$ e atropina ou oximas (tratamento clássico das intoxicações por OFs) é mais efetiva que as suas administrações isoladas em reduzir os danos causados por OFs.

7 DEMAIS TRABALHOS PUBLICADOS DURANTE O DOUTORADO

LUCHESE, C.; PRIGOL, M.; **ACKER, C. I.**; NOGUEIRA, C. W. Antinociceptive effect of butyl (2-phenylethynyl) selenide on formalin test in mice: Evidences for the involvement of serotonergic and adenosinergic systems. Eur. J. Pharmacol. 644, 49-54, 2010.

ACKER, C. I.; SOUZA, A. C. G.; PINTON, S.; DA ROCHA, J. T.; FRIGGI, C. A.; ZANELLA, R.; NOGUEIRA, C. W. Repeated malathion exposure induces behavioral impairment and AChE activity inhibition in brains of rat pups. Ecotoxicol. Environ. Saf. 74, 2310-2315, 2011.

SCHUMACHER, R. F.; ROSARIO, A. R.; SOUZA, A. C. G.; **ACKER, C. I.**; NOGUEIRA, C. W.; ZENI, G. The potential antioxidant activity of 2,3-dihydroselenophene, a prototype drug of 4-aryl-2,3-dihydroselenophenes. Bioorg. Med. Chem. 19, 1418-1425, 2011.

SOUZA, A. C. G.; **ACKER, C. I.**; GAI, B. M.; NETO, J. S. D.; NOGUEIRA, C. W. 2-Phenylethynyl-butyltellurium improves memory in mice. Neurochem. Int. 4, 409-414, 2012.

COSTA, M. D; GAI, B. M.; **ACKER, C. I.**; SOUZA, A. C. G.; BRANDAO, R; NOGUEIRA, C. W. Ebselen reduces hyperglycemia temporarily-induced by diazinon: A compound with insulin-mimetic properties. Chem. Biol. Interact. 197, 80-86, 2012.

LUCHESE, C.; BRANDAO, R.; **ACKER, C. I.**; NOGUEIRA, C. W. 2,2'-Dipyridyl diselenide is a better antioxidant than other disubstituted diaryl diselenides. Mol. Cell. Biochem. 367, 153-163, 2012.

8 REFERÊNCIAS

ABDOLLAHI, M.; RAINBA, A.; SHADNIA, S.; NIKFAR, S.; REZAIE, A. Pesticide and oxidative stress: a review. **Med. Sci. Monitor.** 10, 141-147, 2004a.

ABDOLLAHI, M.; DONYAVI, M.; POURNOURMOHAMMADI, S.; SAADAT, M. Hyperglycemia associated with increased hepatic glycogen phosphorylase and phosphoenolpyruvate carboxykinase in rats following sub chronic exposure to Malathion. **Comp. Biochem. Physiol. (C)** 137, 247-343, 2004b.

ALONZO, H. G. A.; CORRÊA, C. L. Praguicidas. **Fundamentos de toxicologia**, São Paulo: Atheneu, 2002, 437-458 p.

AYGUN, D.; ERENLER, A. K.; KARATAZ, A. D.; BAYDIN, A. Intermediate syndrome following acute organophosphate poisoning: correlation with initial serum levels of muscle enzymes. **Basic Clin. Pharmacol. Toxicol.** 100, 201-204, 2007.

BARBOSA, N. B. V.; ROCHA, J. B. T.; WONDRACEK, D. C.; PEROTTONI, J.; ZENI, G.; NOGUEIRA, C. W. Diphenyl diselenide reduces temporarily hyperglycemia: possible relationship with oxidative stress. **Chem. Biol. Interact.** 163, 230-238, 2006.

BARBOSA, N. B. V.; OLIVEIRA, C.; ARALDI, D.; FOLMER, V.; ROCHA, J. B. T.; NOGUEIRA, C. W. Acute diphenyl diselenide treatment reduces hyperglycemia but does not change delta-aminolevulinate dehydratase activity in alloxan-induced diabetes in rats. **Biol. Pharm. Bull.** 31, 2200-2204, 2008.

BARRY, R. C.; LIN, Y.; WANG, J.; LIU, G.; TIMCHALK, C. A. Nanotechnology-based electrochemical sensors for biomonitoring chemical exposures. **J. Expo. Sci. Environ. Epidemiol.** 19, 1-18, 2009.

BORGES, V. C.; ROCHA, J. B. T.; NOGUEIRA, C. W. Effect of diphenyl diselenide, diphenyl ditelluride and ebselen on cerebral Na^+,K^+-ATPase activity in rats. **Toxicology** 215, 191-197, 2005.

BORGES, L. P.; BRNDÃO, R.; GODÓI, B; NOGUEIRA, C. W.; ZENI, G. Oral administration of diphenyl diselenide protects against cadmium-induced liver damage in rats. **Chem. Biol. Interact.** 171, 15-25, 2008.

BRANDÃO, R.; BORGES, L. P.; DE OLIVEIRA, R.; ROCHA, J. B. T.; NOGUEIRA, C. W. Diphenyl diselenide protects against hematological and immunological alterations induced by mercury in mice. **J. Biochem. Mol. Toxicol.** 22, 311-319, 2008.

BRANDÃO, R.; BORGES, L. P.; NOGUEIRA, C. W. 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.** 47, 1771-1778, 2009.

BUGAJSKI, J.; GADEK-MICHALSKA, A.; BUGAJSKI, A.J. A single corticosterone pretreatment inhibits the hypothalamic-pituitary-adrenal responses to adrenergic and cholinergic stimulation. **J. Physiol. Pharmacol.** 52, 313-324, 2001.

BUSBY-HJERPE, A. L.; CAMPBELL, J. A.; SMITH, J. N.; LEE, S.; POET, T. S.; BARR, D. B.; TIMCHALK, C. Comparative pharmacokinetics of chlorpyrifos versus its major metabolites following oral administration in the rat. **Toxicology** 268, 55-63, 2010.

CERIELLO, A. Acute hyperglycaemia and oxidative stress generation. **Diabet. Med.** 14, 45-49, 1997.

ÇETIN, E.; KANBUR, M.; SILICI, S.; ERASLAN, G. Propetamphos-induced changes in haematological and biochemical parameters of female rats: Protective role of propolis. **Food Chem.Toxicol.** 48, 1806-1810, 2010.

CHAMBERS, J. E.; CARR, R. L.; BOONE, S.; CHAMBERS, H. W. The metabolism of organophosphorus insecticides. **Handbook of Pesticide Toxicology**, 2. Ed., Academic Press, USA, 2001, 919-927 p.

CHEN, D.; SHI, N.; LI, T.; WANG, B. Comparative study of the toxic effects of methamidophos and acephate on intracellular free Ca^{2+} and cAMP concentrations in rat brain tissue. **Toxicology** 191, 34-35, 2003.

COSTA, L. Current issues in organophosphate toxicology. **Clin. Chim. Acta** 306, 1-13, 2006.

DA ROCHA, J. T.; SPERANÇA, A.; NOGUEIRA, C. W.; ZENI, G. Hypolipidaemic activity of orally administered diphenyl diselenide in Triton WR-1339-induced hyperlipidaemia in mice. **J. Pharm. Pharmacol.** 61, 1673-1679, 2009.

DA ROCHA, J. T.; PINTON, S.; MAZZANTI, A.; MAZZANTI, C. M.; BECKEMANN, D. V.; NOGUEIRA, C. W.; ZENI, G. Effects of diphenyl diselenide on lipid profile and

hepatic oxidative stress parameters in ovariectomized female rats. **J. Pharm. Pharmacol.** 63, 663-669, 2011.

DATTA, S.; DHAR, P.; MUKHERJEE, A.; GHOSH, S. Influence of polyphenolic extracts from Enydra fluctuans on oxidative stress induced by acephate in rats. **Food Chem. Toxicol.** 48, 2766-2771, 2010.

DOMINGUES, M. R.; BERNARDI, M. R.; ONO, E. Y. S.; ONO, M.A. Agrotóxicos: Risco à Saúde do Trabalhador Rural. **Rev. Ciências biológicas e da Saúde**, 25, 45-54, 2004.

EATON, D. L.; DAROFF, R. B.; AUTRUP, H.; BRIDGES, J.; BUFFLER, P.; COSTA, L. G.; COYLE, J.; MCKHANN, G.; MOBLEY, W. C.; NADEL, L.; NEUBERT, D.; SCHULTE-HERMANN, R.; SPENCER, P. S. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. **Crit. Rev. Toxicol.** 38, 1-125, 2008.

FORTUNATO, J. J.; AGOSTINHO, F. R.; REUS, G. Z.; PETRONILHO, F. C.; DAL-PIZZOL, F.; QUEVEDO, J. Lipid peroxidative damage on malathion exposure in rats. **Neurotox. Res.** 9, 23-28, 2006.

GHISLEINE, G.; PORCIÚNCULA, L. O.; CIMAROSTI, H.; ROCHA, J. B. T.; SALBEGO, C. G.; SOUZA, D. O. Diphenyl diselenide protects rat hippocampal slices submitted to oxygen-glucose deprivation and diminishes inducible nitric oxide synthase immunocontent. **Brain Res.** 986, 196-199, 2003.

GILLERY, P. Oxidative stress and protein glycation in diabetes mellitus. **Ann. Biol. Clin.** 64, 309-314, 2006.

GOEL, A.; DANI, V.; DHAWAN, D. K. Role of zinc in mitigating the toxic effects of chlorpyrifos on hematological alterations and electron microscopic observations in rat blood. **BioMetals** 19, 483-492, 2006.

GOKCIMEN, A.; GULLE, K.; DEMIRIN, H.; BAYRAM, D.; KOCAK, A.; ALTUNTAS, I. Effects of diazinon at different doses on rat liver and pancreas tissues. **Pest. Biochem. Physiol.** 87, 103-108, 2007.

HALLIWELL, B. Free radicals and antioxidants - quo vadis? **Trends Pharmacol. Sci.** 32,125-130, 2011.

JOSHI, A. K. R.; RAJINI, P. S. Reversible hyperglycemia in rats following acute exposure to

acephate, an organophosphorus insecticide: Role of gluconeogenesis. **Toxicology** 257, 40-45, 2009.

JOSHI, A. K. R.; RAJINI, P. S. Hyperglycemic and stressogenic effects of monocrotophos in rats: Evidence for the involvement of acetylcholinesterase inhibition. **Exp. Toxicol. Pathol.** 64, 115-120, 2012.

KADE, I. J.; BORGES, V. C.; SAVEGNAGO, L.; IBUKUN, E. O.; ZENI, G.; NOGUEIRA, C. W.; ROCHA, J. B. T. Effect of oral administration of diphenyl diselenide on antioxidant status, and activity of delta aminolevulinic acid dehydratase and isoforms of lactate dehydrogenase, in streptozotocin-induced diabetic rats. **Cell Biol. Toxicol.** 25, 415-424, 2009.

KAMATH, V.; RAJINI, P. S. Altered glucose homeostasis and oxidative impairment in pancreas of rats subjected to dimethoate intoxication. **Toxicology** 231, 137-146, 2006.

KHAN, S. M.; KOUR, G. Subacute oral toxicity of chlorpyriphos and protective effect of green tea extract. **Pest. Biochem. Phisiol.** 89, 118-123, 2007.

KOBAYASHI, S. D.; VOYICH, J. M.; DELEO, F. R. Regulation of the neutrophil-mediated inflammatory response to infection. **Microbes Infect.** 5, 1337-1344, 2003.

KOUSBA, A. A.; SULTATOS, L. G.; POET, T. S.; TIMCHALK, C. Comparison of chlorpyrifos-oxon and paraoxon acetylcholinesterase inhibition dynamics: potential role of a peripheral binding site. **Toxicol. Sci.** 80, 239-248, 2004.

LASRAM, M. M.; ANNABI, A. B.; REZG, R.; ELJ, N.; SLIMEN, S.; KAMOUN, A.; EL-FAZAA, S.; GHARBI, N. Effect of short-time malathion administration on glucose homeostasis in Wistar rat. **Pestic. Biochem. Physiol.** 92, 114-119, 2008.

LASRAM, M. M.; ANNABI, A. B.; ELJ, N. E.; SELMI, S.; KAMOUN, A.; EL-FAZAA, S.; GHARBI, N. Metabolic disorders of acute exposure to malathion in adult Wistar rats. **J. Hazard. Mater.** 163, 1052-1055, 2009.

LUCHESE, C.; STANGHERLIN, E. C.; GAY, B. M.; NOGUEIRA, C. W. Antioxidant effect of diphenyl diselenide on oxidative damage induced by smoke in rats: Involvement of glutathione. **Ecotoxicol. Environ. Saf.** 72, 248-254, 2009.

LUCHESE, C.; NOGUEIRA, C. W. Diphenyl diselenide in its selenol form has dehydroascorbate reductase and glutathione S-transferase-like activity dependent on the

glutathione content. **J. Pharm. Pharmacol**. 62, 1146-1151, 2010.

LUKASZEWICZ-HUSSAIN, A. Role of oxidative stress in organophosphate insecticide toxicity - Short review. **Pestic. Biochem. Physiol.** 98, 145-150, 2010.

MAHAJNA, M.; QUISTAD, B. G.; CASIDA, J. E. Acephate insecticide toxicity: safety conferred by inhibition of the bioactivating carboxyamidase by the metabolite methamidophos. **Chem. Res. Toxicol.** 10, 64-69, 1997.

MEHTA, A.; VERMA, R. S.; SRIVASTAVA, N. Chlorpyrifos-induced DNA damage in rat liver and brain. **Environ. Mol. Mutagen.** 49, 426-433, 2008.

NOGUEIRA, C. W.; BORGES, V. C.; ZENI, G.; ROCHA, J. B. T. Organochalcogens effects on δ-aminolevulinate dehydratase activity from human erythrocytic cells in vitro. **Toxicology** 191, 169-178, 2003a.

NOGUEIRA, C. W.; MEOTTI, F. C.; CURTE, E.; PILISSÃO, C.; ZENI, G.; ROCHA, J. B. T. Investigations into the potential neurotoxicity induced by diselenides in mice and rats. **Toxicology** 183, 29-37, 2003b.

NOGUEIRA, C. W.; ZENI, G.; ROCHA, J. B. T. Organoselenium and organotellurium compounds: toxicology and pharmacology. **Chem. Rev.** 104, 6255-6286, 2004.

NOGUEIRA, C. W.; ROCHA, J. B. T. Diphenyl diselenide: a janus-faced molecule. **J. Braz. Chem. Soc.** 21, 2055-2017, 2010.

PANEMANGALORE, M.; DOWLA, H. A.; BYERS, M. E. Occupational exposure to agricultural chemicals: effect on the activities of some enzymes in the blood of farm workers. **Int. Arch. Occup. Environ. Health** 72, 84-88, 1999.

POSSAMAI, F. P.; FORTUNATO, J. J.; FEIER, G.; AGOSTINHO, F. R.; QUEVEDO, J.; WILHELM Filho, D.; DAL-PIZZOL, F. Oxidative stress after acute and sub-chronic malathion intoxication in Wistar rats. **Environ. Toxicol. Pharmacol.** 23, 198-204, 2007.

PRIGOL, M. **Ação convulsivante do disseleneto de difenila em ratos**: estudo dos mecanismos neuroquímicos e toxicocinética. 2010. Tese (Doutorado em Ciências Biológicas: Bioquímica Toxicológica) – Universidade Federal de Santa Maria, Santa Maria, 2010.

PRIGOL, M.; WILHELM, E. A.; STANGHERLIN, E. C.; BARANCELLI, D. A.; NOGUEIRA, C. W.; ZENI, G. Diphenyl diselenide-induced seizures in rat pups: possible interaction with glutamatergic system. **Neurochem. Res.** 33, 996-1004, 2008.

PRIGOL, M.; LUCHESE, C.; NOGUEIRA, C. W. Antioxidant effect of diphenyl diselenide on oxidative stress caused by acute physical exercise in skeletal muscle and lungs of mice. **Cell Biochem. Funct.** 27, 216-222, 2009a.

PRIGOL, M.; SCHUMACHER, R. F.; NOGUEIRA, C. W.; ZENI, G. Convulsant effect of diphenyl diselenide in rats and mice and its relationship to plasma levels. **Toxicol. Lett.** 189, 35-39, 2009b.

RAHIMI, R.; NIKFAR, S.; ABDOLLAHI, M. Increased morbidity and mortality in acute human organophosphate-poisoned patients treated by oximes: a meta-analysis of clinical trials. **Hum. Exp. Toxicol.** 25, 157-162, 2006.

RAHMAN, M. F.; MAHBOOB, M.; DANADEVI, K.; SALEHA BANU, B.; GROVER, P. Assessment of genotoxic effects of chloropyriphos and acephate by the comet assay in mice leucocytes. **Mutat. Res.** 516, 139-147, 2002.

REZG, R.; MORNAGUI, B.; KAMOUN, A.; EL-FAZAA, S.; GHARBI, N. Effect of subchronic exposure to malathion on metabolic parameters in the rat. **C R Biol.** 330, 143-147, 2007.

SAVOLAINEN, K. Understanding the toxic action of organophosphates. **Handbook of Pesticide Toxicology**, 2. Ed., Academic Press, USA, 2001, 1013-1043 p.

SHADNIA, S.; AZIZI, E.; HOSSEINI, R.; KHOEI, S.; FOULADDEL, S.; PAJOUMAND, A.; JALALI, N.; ABDOLLAHI, M. Evaluation of oxidative stress and genotoxicity in organophosphorus insecticide formulators. **Hum. Exp. Toxicol.** 24, 439-445, 2005.

SHARMA, Y.; BASHIR, S.; IRSHAD, M.; GUPTA, S. D.; DOGRA, T. D. Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. **Toxicology** 206, 49-54, 2005.

SINGH, S.; KUMAR, V.; THAKUR, S.; BANERJEE, B. D.; CHANDNA, S.; RAUTELA, R. S.; GROVER, S. S.; RAWAT, D. S.; PASHA, S. T.; JAIN, S. K.; ICHHPUJANI, R. L.; RAI, A. DNA damage and cholinesterase activity in occupational workers exposed to pesticides. **Environ. Toxicol. Pharmacol.** 31, 278-285, 2011.

SINITOX - Sistema Nacional de Informações Tóxico-Farmacológicas. **Casos Registrados de Intoxicação Humana, de Intoxicação Animal e de Solicitação de Informação por Agente Tóxico.** Brasil, 2009. Disponível em: http://www.fiocruz.br/sinitox_novo/media/tab04_brasil_2009.pdf. Acesso em: 03 out. 2011.

SOLTANINEJAD, K.; ABDOLLAHI, M. Current opinion on the science of organophosphate pesticides and toxic stress: a systematic review. **Med. Sci. Monit.** 15, 75-90, 2009.

TEIMOURI, F.; AMIRKABIRIAN, N.; ESMAILY, H.; MOHAMMADIRAD, A.; ALIAHMADI, A.; ABDOLLAHI, M. Alteration of hepatic cells glucose metabolism as a non-cholinergic detoxication mechanism in counteracting diazinon-induced oxidative stress. **Hum. Exp. Toxicol.** 25, 697-703, 2006.

THRASHER, J. D.; MADISON, R.; BROUGHTON, A. Immunologic abnormalities in human exposed to chlorpyrifos: preliminary observations. **Arch. Environ. Health** 48, 89-93, 1993.

TIAN, Y.; ISHIKAWA, H.; YAMAGUCHI, T.; YAMAUCHI, T.; YOKOYAMA, K. Teratogenicity and developmental toxicity of chlorpyrifos maternal exposure during organogenesis in mice. **Reprod. Toxicol.** 20, 267-271, 2005.

TOMLIN, C. The pesticide manual. **The Royal Society of Chemistry**,10. ed., Surrey, 1995, 1341 p.

VERMA, R. S.; SRIVASTAVA, N. Effect of chlorpyrifos on thiobarbituric acid reactive substances, scavenging enzymes and glutathione in rat tissues. **Indian J. Biochem. Biophys.** 40, 423-428, 2003.

VERMA, R. S.; MEHTA, A.; SRIVASTAVA, N. *In vivo* chlorpyrifos induced oxidative stress: Attenuation by antioxidant vitamins. **Pest. Biochem. Physiol.** 88, 191-196, 2007.