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AVALIAÇÃO DOS EFEITOS DO TRATAMENTO CRÔNICO COM NEUROLÉPTICOS E SUA INTERAÇÃO COM SUBSTÂNCIAS POTENCIALMENTE ANTIOXIDANTES SOBRE PARÂMETROS DE ESTRESSE OXIDATIVO NO FÍGADO E RIM DE RATOS

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS, Brasil
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AVALIAÇÃO DOS EFEITOS DO TRATAMENTO CRÔNICO COM NEUROLÉPTICOS E SUA INTERAÇÃO COM SUBSTÂNCIAS POTENCIALMENTE ANTIOXIDANTES SOBRE PARÂMETROS DE ESTRESSE OXIDATIVO NO FÍGADO E RIM DE RATOS

por

Cristiane Lenz Dalla Corte

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

Orientador: Prof. Dr. Félix Alexandre Antunes Soares

Co-orientador: Prof. Dr. João Batista Teixeira da Rocha

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elaborada por

Cristiane Lenz Dalla Corte

Como requisito parcial para a obtenção de grau de **Mestre em Bioquímica Toxicológica**

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SUMÁRIO

LISTA DE ABREVIATURAS

ALA – ácido 5’-aminolevulínico

δ-ALA-D – delta aminolevulinato desidratase

ALT – alanina aminotransferase

ATP – adenosina trifosfato

AST – aspartato aminotransferase

CAT – catalase

CYP – citocromo P450

DCFH – diclorofluoresceína

DO – discinesia orofacial

DT – discinesia tardia

DTT – DL-ditiotreitol

EROS – espécies reativas de oxigênio

GABA – ácido gama aminobutírico

GAD – ácido glutâmico descarboxilase

GPx – glutationa peroxidase

GSH – glutationa reduzida

GSSG – glutationa oxidada

MDA – ácido malondialdeído

NMDA – *N*-metil-*D*-aspartato

SOD – superóxido dismutase

TBARS – espécies reativas ao ácido tiobarbitúrico

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APRESENTAÇÃO

No item **INTRODUÇÃO**, está descrita uma sucinta revisão bibliográfica sobre os temas trabalhados nesta dissertação.

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de artigos, os quais se encontram no item **ARTIGOS CIENTÍFICOS**. As seções Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se nos próprios artigos e representam a íntegra deste estudo.

Os itens, **DISCUSSÃO E CONCLUSÕES**, encontram-se no final desta dissertação, apresentam interpretações e comentários gerais sobre os artigos científicos contidos neste trabalho.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações que aparecem nos itens **INTRODUÇÃO, DISCUSSÃO e CONCLUSÕES** desta dissertação.

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Bioquímica Toxicológica
Universidade Federal de Santa Maria, RS, Brasil

AVALIAÇÃO DOS EFEITOS DO TRATAMENTO CRÔNICO COM NEUROLÉPTICOS E SUA INTERAÇÃO COM SUBSTÂNCIAS POTENCIALMENTE ANTIOXIDANTES SOBRE PARÂMETROS DE ESTRESSE OXIDATIVO NO FÍGADO E RIM DE RATOS

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LOCAL E DATA DA DEFESA: Santa Maria, Março de 2008.

O tratamento com drogas neurolépticas tem sido associado a efeitos colaterais como a discinesia tardia (DT) e o dano hepático. Apesar dos inúmeros casos de hepatotoxicidade após a administração de neurolépticos, são escassos os dados na literatura a respeito desses efeitos e o mecanismo exato pelo qual neurolépticos induzem hepatotoxicidade permanece incerto. Da mesma forma, existem poucos estudos relatando os efeitos dos neurolépticos sobre o rim. Dessa forma, o primeiro objetivo deste trabalho foi avaliar os efeitos da exposição crônica à flufenazina em fígado e rim de ratos bem como o efeito protetor do disseleneto de difenila sobre o dano induzido por flufenazina (artigo 1). O tratamento prolongado com flufenazina causou um aumento na peroxidação lipídica no fígado e no rim, uma diminuição na atividade da SOD hepática, e um aumento na atividade da CAT hepática. O disseleneto de difenila foi capaz de proteger o fígado e o rim da peroxidação lipídica, melhorou a atividade da SOD no fígado, e preveniu o aumento na atividade da CAT no fígado. O tratamento com disseleneto de difenila não afetou a atividade da δ-ALA-D, mas a flufenazina e/ou em combinação com disseleneto de difenila demonstrou ter efeito inibitório sobre a atividade da δ-ALA-D no fígado e no rim. O segundo objetivo deste estudo foi determinar se o tratamento com haloperidol (HP), valeriana ou a associação de ambas as drogas pode alterar as funções hepáticas e renais (artigo 2). A valeriana não afetou nenhum parâmetro de estresse oxidativo no fígado e no rim dos ratos. O HP apenas aumentou a depleção de glutationa (GSH) no fígado, mas não no rim. Entretanto, quando o HP foi associado com a valeriana, um aumento na peroxidação lipídica e produção de espécies reativas foram observados no tecido hepático. HP e valeriana quando administrados independentemente não afetaram a atividade da δ-ALA-D hepática e renal, contudo, quando estas drogas foram administradas concomitantemente provocaram uma inibição da atividade da δ-ALA-D hepática. A atividade da aspartato aminotransferase (AST) do soro não foi alterada por nenhum dos tratamentos. No entanto, a atividade da alanina aminotransferase (ALT) do soro estava aumentada nos

grupos tratados com HP e HP mais flufenazina. Juntos estes resultados indicam uma relação entre o tratamento com flufenazina e o estresse oxidativo, e também apontam para o papel protetor do disseleneto de difenila no dano oxidativo induzido por flufenazina no fígado. Nossos dados também sugerem interações adversas no tratamento com haloperidol e valeriana, ocasionando dano hepático associado ao estresse oxidativo.

***Palavras-chave*:** flufenazina; selênio; disseleneto de difenila; haloperidol; *Valeriana officinalis*; interação planta medicinal-fármaco; estresse oxidativo; TBARS; δ-ALA-D.

ABSTRACT

Dissertation of Master's Degree
Graduate Course in Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil

ASSESSMENT OF THE EFFECTS OF CHRONIC TREATMENT WITH NEUROLEPTICS AND THEIR INTERACTION WITH POTENTIALLY ANTIOXIDANTS SUBSTANCES ON OXIDATIVE STRESS PARAMETERS IN LIVER AND KIDNEY OF RATS

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PLACE AND DATE OF THE DEFENSE: Santa Maria, March, 2008.

Treatment with neuroleptic drugs has been associated to side effects like tardive diskynesia and hepatic damage. In spite of the several reports of hepatotoxicity after neuroleptic administration, few data are available in the literature about these effects and the precise mechanisms by which neuroleptics induce hepatotoxicity remain unclear. In the same way, there are few studies about the effects of neuroleptics on kidney. In this way, the first aim of the present work was to assess the effects of chronic exposure to fluphenazine in liver and kidney of rats, as well as the protective effect of diphenyl diselenide on the fluphenazine-induced damage (article 1). Long-term treatment with fluphenazine caused an increase in lipid peroxidation levels in liver and kidney homogenates, a decrease in hepatic SOD activity, and an increase in hepatic CAT activity. Diphenyl diselenide was able to protect liver and kidney from lipid peroxidation, ameliorate SOD activity in liver, and prevent the increase in hepatic CAT activity. Diphenyl diselenide treatment did not affect δ-ALA-D activity, but fluphenazine and/or in combination with diphenyl diselenide showed an inhibitory effect on δ-ALA-D activity in liver and kidney. The second objective of this study was to determine whether the treatment with haloperidol (HP), valerian or both in association impairs liver or kidney functions (article 2). Valerian did not affect oxidative stress parameters in the liver or kidney of rats. HP only increased glutathione (GSH) depletion in liver, but not in kidney. However, when HP was associated with valerian, an increase in lipid peroxidation levels and reactive species production was observed in the hepatic tissue. HP and valerian when administered independently did not affect the activity of hepatic and renal δ-ALA-D, however, these drugs administered concomitantly provoked an inhibition of hepatic δ-ALA-D activity. Serum aspartate aminotransferase (AST) activity was not altered by any treatment. However, serum alanine aminotransferase (ALT) activity was higher in the HP group and HP plus valerian group. Taken together, these results indicate the relationship between the treatment with flufenazine and the oxidative stress, and also point to the protective role

of diphenyl diselenide on the oxidative damage induced by fluphenazine in liver. Our data also suggest adverse interactions between haloperidol and valerian treatments causing hepatic damage related to oxidative stress.

Keywords**:** fluphenazine; selenium; diphenyl diselenide; haloperidol; *Valeriana officinalis*; herb-drug interaction; oxidative stress; TBARS; δ-ALA-D.

1. INTRODUÇÃO

1.1. Neurolépticos

1.1.1. Histórico

Neurolépticos ou antipsicóticos são drogas utilizadas no tratamento de doenças psiquiátricas graves, assim como, das psicoses e da mania. A descoberta do primeiro antipsicótico, a clorpromazina, deu-se, em parte, ao acaso em 1950 por Laborit. No entanto as primeiras tentativas de tratar as doenças mentais com clorpromazina foram feitas por Delay e Deniker em 1952. Apesar do surgimento dos neurolépticos ter representado um dos mais importantes avanços na história da psicofarmacologia e psiquiatria, estes fármacos possuem eficácia comprometida devido ao surgimento de efeitos colaterais extrapiramidais como a Discinesia Tardia (DT) e o Parkisonismo. Atualmente, os esforços concentram-se na busca por drogas com menos efeitos extrapiramidais e mais eficientes nos tratamento dos sintomas negativos da esquizofrenia. Estas drogas são denominadas neurolépticos atípicos dentre os quais o principal é a clozapina (Goodman, 2004; Silva, 2006).

As drogas antipsicóticas são agrupadas em antipsicóticos convencionais (ex.: clorpromazina, flufenazina, haloperidol) e antipsicóticos atípicos (ex.:clozapina, olanzapina). As diferenças entre ambos os grupos são definidas em termos clínicos e farmacológicos. Clinicamente, drogas antipsicóticas atípicas causam menos efeitos extrapiramidais, e são mais efetivos que as drogas convencionais em tratar os sintomas negativos da esquizofrenia (ex.: isolamento social, embotamento afetivo) (Konradi e Heckers, 2003). Farmacologicamente, os antipsicóticos convencionais, tais como o haloperidol, tem maior afinidade por receptores D_2 (Levinson, 1991), enquanto antipsicóticos atípicos como a clozapina tem afinidade por múltiplos sistemas de receptores, incluindo os receptores D_2 (Remington e Chong, 1999). As drogas antipsicóticas atuam primariamente sobre os sistemas dopaminérgicos e serotoninérgicos (5-HT), e embora elas tenham efeitos diretos sobre o sistema glutamatérgico, em geral estes efeitos são pequenos. Entretanto, através da sua interação

com sistemas monoaminérgicos, as drogas antipsicóticas podem modular a função glutamatérgica através de um potente mecanismo indireto (Leveque e cols., 2000).

Estudos relataram que a potência clínica das drogas antipsicóticas convencionais está diretamente relacionada com a sua afinidade por receptores dopaminérgicos D_2 (Seeman e Lee, 1975; Creese e cols., 1976; Seeman e Van Tol, 1993). Drogas antipsicóticas convencionais inibem os receptores D_2 provocando, incialmente, no neurônio pré-sináptico, aumento na produção e liberação de dopamina, por aumento de atividade da enzima tirosina hidroxilase, na tentativa de vencer o bloqueio (Silva, 2006). Os neurolépticos fenotiazínicos que apresentam um grupamento piperazínico constituem alguns dos antipsicóticos mais potentes, é o caso da flufenazina. Estes compostos apresentam atividade anticolinégica relativamente fraca, têm menor tendência para produzir sedação e causam menos efeitos autonômicos, no entanto possuem um acentuado risco de induzir efeitos extrapiramidais. Outra classe de neurolépticos, as butirofenonas incluem o haloperidol. São drogas antipsicóticas potentes, e frequentemente produzem sintomas extrapiramidais, possívelmente em decorrência de sua baixa atividade anticolinérgica (Goodman, 2004; Silva, 2006).

1.1.2. Discinesia Tardia

Os efeitos colaterais mais prevalentes e incômodos associados ao uso de neurolépticos envolvem o sistema motor extrapiramidal. O surgimento destes efeitos é mais pronunciado nas drogas com menor ação anticolinérgica como butirofenonas (haloperidol) e fenotiazinas piperazínicas (flufenazina). A DT pode surgir após meses ou até anos após o uso de neurolépticos (Crane, 1973; Jeste e cols., 1979; Casey, 1985; Glazer e cols., 1990), e manifesta-se através de movimentos orofaciais involuntários e estereotipados, que pioram com a suspensão do tratamento. A DT ocorre em 20-25% dos pacientes que recebem tratamento com neurolépticos clássicos. Esta razão aumenta consideravelmente com a idade, uma prevalência acima de 50% foi descrita pra pacientes com mais de 50 anos (Kane e Smith, 1982; Woerner e cols., 1991; Yassa e Jeste, 1992).

Várias hipóteses têm sido propostas para explicar a fisiopatologia da DT, e é possível que mecanismos diferentes estejam envolvidos no seu desenvolvimento. Uma das hipóteses que têm recebido grande atenção nas últimas duas décadas é a da hipersensibilidade dopaminérgica. Segundo esta hipótese, a DT é resultante de uma supersensibilidade dopaminérgica devido ao bloqueio crônico dos receptores dopaminérgicos pelos neurolépticos, em locais relacionados ao controle dos movimentos (Klawans e Rubovits, 1972; Burt e cols., 1977; Rubinstein e cols., 1990). Em resposta a este bloqueio crônico, há um aumento compensatório do número e sensibilidade dos receptores dopaminérgicos levando a um estado hiperdopaminérgico e a manifestações clínicas como, por exemplo, a DT (Cavallaro e Smeraldi, 1995; Kane, 1995). Essa hipótese, no entanto, possui algumas limitações, pois, não consegue explicar porque a DT se desenvolve apenas em alguns pacientes, porque demora anos para se desenvolver, porque persiste mesmo após a interrupção do tratamento, e porque alguns fatores como idade, gênero, diabetes mellitus, etc. aumentam seu risco (Smith, 1988; Sachdev e cols., 1999).

Uma das primeiras hipóteses propostas para a DT diz respeito a alterações na transmissão gabaérgica provocada por neurolépticos nos glânglios da base (Fibiger e Lloyd, 1984). Esta hipótese baseia-se em relatos de que macacos e ratos com movimentos orofaciais induzidos por neurolépticos apresentavam diminuição na atividade da enzima glutamato descarboxilase (GAD) na *substantia nigra*, no *globus palidus* e no núcleo subtalâmico (Gunne e Haggstrom, 1983; Gunne e cols., 1984; Johansson e cols., 1990). Estudos em ratos onde agonistas gabaérgicos inibiram o desenvolvimento de movimentos de mascar no vazio induzidos por neurolépticos também corroboram com esta hipótese (Kaneda e cols., 1992; Gao e cols., 1994).

Outra hipótese proposta é da excitotoxicidade. Esta hipótese propõe que a utilização em longo prazo de neurolépticos aumenta a liberação de glutamato a partir dos terminais córtico-estriatais levando a excitotoxicidade estriatal (De Keyser, 1991). O envolvimento da excitotoxicidade no dano neuronal agudo já é bem descrito, no entanto o exato mecanismo para a excitotoxicidade na neurodegeneração crônica e DT permanece a ser esclarecido. Uma possibilidade é o prejuízo do metabolismo energético

o qual é um dos efeitos dos neurolépticos convencionais (Burkhardt e cols., 1993). A interrupção da síntese de ATP leva a diminuição do potencial de membrana que facilita a ativação de receptores NMDA devido ao menor bloqueio do Mg^{2+} dependente da voltagem. Dessa forma, níveis fisiológicos de glutamato podem induzir um influxo de Ca^{2+} excessivo o qual desencadeia uma cascata de reações tóxicas levando à morte celular (Novelli e cols., 1988).

Um mecanismo que vem ganhando reconhecimento nos últimos anos é a hipótese dos radicais livres. Os neurolépticos induzem um aumento no "turnover" de dopamina (See, 1991) o que pode levar a superprodução de espécies reativas de oxigênio (EROS) (Andreassen e Jorgensen, 2000). Estes níveis aumentados de EROS podem afetar negativamente a neurotransmissão e a viabilidade celular (Andreassen e Jorgensen, 2000). Vários estudos dão suporte a esta hipótese: 1) relatos da redução nos ácidos graxos essenciais em fosfolipídios no plasma de pacientes com DT (Horrobin e cols., 1989); 2) aumento nos níveis de peroxidação lipídica no fluído cerebroespinhal de pacientes com DT (Lohr e cols., 1990); 3) possíveis efeitos benéficos da vitamina E e outros antioxidantes na DT (Egan e cols., 1997; Burger e cols., 2003; Burger e cols., 2005; Burger e cols., 2006); e 4) o papel da idade, diabetes, fumo, e dano cerebral como fatores de risco (Sachdev e cols., 1999; Burger e cols., 2004).

1.1.3. Hepatotoxicidade

A estratégia para a escolha de um agente antipsicótico deve tomar em conta a tolerância hepática com base na significante incidência de desordens hepáticas entre a população (presença de fatores de risco como alcoolismo, drogas de abuso, polimedicação incluindo drogas potencialmente hepatotóxicas, etc). Nos Estados Unidos, em 2003, a injúria hepática induzida por drogas foi responsável por mais de 50 % dos casos de falência hepática aguda (Lee, 2003). A lista de fármacos capazes de provocar efeitos colaterais hepáticos inclui mais de mil medicamentos, dos quais 16% são drogas neuropsiquiátricas incluindo os neurolépticos (Dumortier e cols., 2002). Elevações da atividade de enzimas hepáticas ocorrem frequentemente com drogas fenotiazínicas (freqüência avaliada em 20%), mas também com outras classes de

agentes neurolépticos. Por outro lado, a hepatite clínica é mais raramente descrita para drogas neurolépticas como as fenotiazínas (0,1-1 %) ou como o haloperidol (0,002 %) (Dumortier e cols., 2002).

O mecanismo exato pelo qual neurolépticos induzem hepatotoxicidade permanece incerto (Selim e Kaplowitz, 1999; Dumortier e cols., 2002). Uma substância pode ser intrinsecamente hepatotóxica, ou então pode dar origem a um metabólito tóxico, que o tecido hepático pode ter ou não capacidade de depurar. O citocromo P450, uma família de enzimas largamente envolvida em reações oxidativas no metabolismo das drogas, é responsável pela produção de intermediários altamente reativos. Assim xenobióticos podem sofrer bioativação em eletrófilos e radicais livres e provocar toxicidade pela modificação de macromoléculas celulares (Kaplowitz, 1996; Park e cols., 2005). Dessa forma, os metabólitos das drogas podem participar de uma série de reações químicas, como depleção da glutationa (GSH), ligação covalente com proteínas, lipídios, ou ácidos nucléicos, ou indução de peroxidação lipídica. Todos estes eventos têm efeitos diretos sobre as organelas celulares e podem também influenciar indiretamente as organelas através da ativação e inibição de quinases sinalizadoras, fatores de transcrição e da expressão gênica (Park e cols., 2005). O estresse intracelular resultante leva à morte celular causada tanto por apoptose quanto por necrose (Kaplowitz, 2000; 2002). A morte do hepatócito é o principal evento que leva à injúria hepática, embora as células endoteliais sinusoidais (DeLeve e cols., 1996) e o epitélio do ducto biliar (Odin e cols., 2001) também possam ser alvos.

Alguns fatores podem aumentar o risco de hepatotoxicidade como o uso concomitante de compostos que causam indução ou inibição do citocromo P450 hepático (Gopaul, 2003) o que pode interferir no metabolismo e eliminação dos fármacos. A proliferação de terapias alternativas e produtos naturais, por exemplo, podem ter conseqüências deletérias. Fitoterápicos são considerados equivocadamente pela população em geral como medicamentos seguros (Eisenberg e cols., 1998; Haller e cols., 2002), no entanto, interações indesejáveis podem ocorrer entre fitoterápicos e drogas convencionais e, portanto, deve se ter cautela com esse tipo de associação (Fugh-Berman e Ernst, 2001).

1.2. Estresse Oxidativo

O balanço entre substâncias pró-oxidantes e antioxidantes é crucial para a sobrevivência e funcionamento dos organismos aeróbicos. Um desequilíbrio favorecendo pró-oxidantes e/ou desfavorecendo antioxidantes é denominado estresse oxidativo sendo potencialmente nocivo (Sies, 1986).

As substâncias pró-oxidantes são naturalmente formadas como produtos do metabolismo aeróbico, mas durante condições patológicas estas são produzidas em níveis elevados. Radicas livres podem ser definidos como moléculas ou fragmentos de moléculas contendo um ou mais elétrons desemparelhados em orbitais atômicos ou moleculares (Halliwell e Gutteridge, 1999). Os elétrons desemparelhados usualmente conferem um considerável grau de reatividade aos radicais livres. Radicais derivados de oxigênio representam a mais importante classe de espécies radicais geradas em sistemas vivos (Miller e cols., 1990). Os passos intermediários da redução do oxigênio consistem na formação do radical ânion superóxido, peróxido de hidrogênio e radical hidroxila correspondendo aos passos de redução por um, dois e três elétrons, respectivamente (Halliwell e Gutteridge, 1999). Os radicais de oxigênio também podem ocorrer como radicais alquila e peroxila, (ex.: em lipídios). Outro radical, o óxido nítrico, pode reagir com o radical ânion superóxido formando o ânion peroxinitrito, o qual é altamente reativo (Sies, 1997).

As estratégias de defesa fisiológicas e farmacológicas contra as substâncias pró-oxidantes consistem em três categorias: prevenção, interceptação e reparo. A primeira linha de defesa contra as espécies reativas é a prevenção contra a sua formação por meios físicos ou bioquímicos. Na segunda linha de defesa, e interceptação, encontram-se antioxidantes enzimáticos e não-enzimáticos. As defesas antioxidantes enzimáticas incluem as enzimas supéroxido dismutase (SOD), glutationa peroxidase (GPx), catalase (CAT). Os antioxidantes não enzimáticos são representados por ácido ascórbico (vitamina C), α-tocoferol (vitamina E), glutationa (GSH), carotenóides e flavonóides (Sies, 1997; Valko e cols., 2007). A proteção contra os efeitos do estresse oxidativo

também pode se dar pelo reparo do dano uma vez que este tenha ocorrido (reparo do DNA, modificações no "turnover" de lipídios, proteólise) (Sies, 1993).

As EROS são bem conhecidas por desempenharem um papel duplo como espécies deletérias e benéficas. Os efeitos benéficos das EROS ocorrem em concentrações baixas a moderadas e envolvem papeis fisiológicos na resposta celular à noxia, como por exemplo, na defesa contra agentes infecciosos, no funcionamento de diversas vias de sinalização celular, e na indução de resposta mitogênica (Valko e cols., 2007). Em contraste, em altas concentrações as EROS podem danificar lipídios das células, proteínas ou DNA, inibindo a sua função normal. Devido a isso, o estresse oxidativo tem sido implicado em várias doenças bem como no processo de envelhecimento (Kovacic e Jacintho, 2001; Valko e cols., 2006; 2007).

1.3. Disseleneto de Difenila

Desde a descoberta da presença do elemento selênio, um calcogênio, no centro ativo das enzimas antioxidantes glutationa peroxidase (GPx) e glutationa peroxidase de hidroperóxidos lipídicos (PHGPx), os compostos orgânicos de selênio vêm despertando grande interesse (Rotruck e cols., 1973; Nogueira e cols., 2004). Devido as possíveis aplicações no tratamento de doenças, novos compostos orgânicos de selênio com atividade mimética da GPx passaram a ser sintetizados e estudados (Parnham e Graf, 1991; Mugesh e cols., 2001; Nogueira e cols., 2004).

O Ebselen (2-fenil-1,2-benzisoselenazol-3[2H]-ona) é um composto orgânico de selênio não tóxico que tem sido extensivamente estudado na última década. O interesse particular neste composto é sua atividade mimética da GPx, especialmente da PHGPx (Wendel e cols., 1984; Müller e cols., 1985; Nogueira e cols., 2002; Klotz e cols., 2003). Além da atividade antioxidante, o ebselen demonstrou possuir propriedades antiinflamatória, antinociceptiva, neuroprotetora e anti-úlcera em vários modelos animais (Maiorino e cols., 1992; Nogueira e cols., 2004). O fígado é outro alvo terapêutico dos compostos orgânicos de selênio. O Ebselen protegeu contra o dano hepático induzido por paracetamol, CCl_4, lipopolissacarídio e *Propionibacterium acnes*,

etanol, e injúria por isquemia-reperfusão (Li e cols., 1994; Ozaki e cols., 1997; Kono e cols., 2001; Koyanagi e cols., 2001; Wasser e cols., 2001).

O disseleneto de difenila, outro composto orgânico de selênio, demonstrou ter maior atividade tiol-peroxidase que o Ebselen (Wilson e cols., 1989), além de ser menos tóxicos a roedores (Meotti e cols., 2003; Nogueira e cols., 2003a). O disseleneto de difenila também possui potenciais antinociceptivo e antiflamatório melhores que o ebselen (Nogueira e cols., 2003b). O disseleneto de difenila foi testado em vários modelos de neuroproteção apresentando bons resultados (Ghisleni e cols., 2003; Nogueira e cols., 2004). Em um modelo agudo de discinesia orofacial (DO), o disseleneto de difenila protegeu contra a DO e a peroxidação lipídica em cérebro causada pela administração de reserpina em ratos (Burger e cols., 2004). Da mesma forma outro estudo demonstrou a proteção do disseleneto de difenila contra a DO em um tratamento agudo com haloperidol (Burger e cols., 2006). Além disso, pré- e pós-tratamentos com disseleneto de difenila foram efetivos em proteger contra o dano hepático induzido por 2-nitropropano (Borges e cols., 2005; 2006).

O mecanismo catalítico para a ação dos compostos orgânicos de selênio é a interação direta com tióis de baixo peso molecular oxidando-os a dissulfetos ao mesmo tempo em que decompõem H_2O_2 (Maiorino e cols., 1988; Nogueira e cols., 2004). Embora a atividade do tipo tiol-peroxidase dos compostos orgânicos de selênio seja importante para suas propriedades antioxidantes, também pode contribuir para suas propriedades toxicológicas devido à oxidação de proteínas e metabólitos tióis importantes. No caso de enzimas isto pode resultar na perda da atividade catalítica (Nogueira e cols., 2004). Um exemplo disso é a enzima δ-aminolevulinato desidratase (δ-ALA-D) uma enzima sulfidrílica extremamente sensível a agentes oxidantes (Barbosa e cols., 1998; Folmer e cols., 2002; 2003; Soares e cols., 2003; Santos e cols., 2005) que catalisa a condensação de duas moléculas do ácido 5'-aminolevulínico (ALA) para formar o porfibilinogênio. Trabalhos demonstram que o tratamento agudo com o composto disseleneto de difenila inibe a atividade da enzima δ-ALA-D devido à oxidação dos resíduos cisteinil presentes no sítio ativo da enzima (Farina e cols., 2002).

1.4. *Valeriana officinalis*

A valeriana *(Valeriana officinalis* L., Valerianaceae) é uma das plantas medicinais mais utilisadas em todo o mundo (Blumenthal, 2003; Gutierrez e cols., 2004). É conhecida e utilizada há séculos devido a suas propriedades calmante, sedativa, ansiolítica, entre outras (Houghton, 1999; Stevinson e Ernst, 2000; Krystal e Ressler, 2001). Os extratos de valeriana são vendidos como suplementos dietéticos e estiveram entre os 10 suplementos fitoterápicos mais vendidos nos Estados Unidos em 2002 (Blumenthal, 2003).

Atualmente não existe concordância no meio científico quanto ao mecanismo pelo qual a valeriana ou seus compostos, exerce sua atividade sedativa, ou os compostos responsáveis por esta atividade. Diversos estudos apontam para um mecanismo de ação gabaérgico para esta planta. Valeriana pode interagir com receptores $GABA_A$ ativando-os (Mennini e cols., 1993; Cavadas e cols., 1995; Ortiz e cols., 2004). Também parece diminuir a degradação do ácido gama aminobutírico (GABA) (Houghton, 1999). O aumento da concentração de GABA na fenda sináptica é um fator responsável pelas propriedades sedativas da valeriana. Extratos de valeriana e ácido valerênico parecem também ter efeito agonista parcial sobre receptores serotoninérgicos (Dietz e cols., 2005). Além dos seus efeitos sedativos, um trabalho recente demonstrou que o extrato de *V. officinalis* possui atividade antioxidante em baixas concentrações em um modelo *in vitro* (Rocha e cols., dados não publicados).

Dados recentes demonstraram o efeito indutor da valeriana sobre as enzimas citocromo P450 3A4 e 2D6 em culturas de hepatócitos de humanos (Hellum e cols., 2006). Este efeito da valeriana sobre a atividade das enzimas citocromo P450 é particularmente importante, pois pode afetar a disponibilidade de drogas convencionais quando valeriana e a droga forem usadas concomitantemente. Vários relatos de casos clínicos indicaram que a valeriana pode causar alterações nas funções hepáticas (Chan, 1998). Em alguns desses relatos de possível toxicidade hepática da valeriana, o consumo foi geralmente crônico e, em alguns casos, outras plantas também foram

consumidas. Acredita-se que problemas hepáticos associados ao uso agudo de valeriana sejam improváveis, no entanto, é possível que a valeriana em longo prazo, sozinha ou em associação com outras plantas ou outras drogas possa causar hepatotoxicidade (Chan e cols., 1995; Willey e cols., 1995; Chan, 1998).

2. OBJETIVOS

Objetivo Geral

Avaliar os efeitos de tratamentos crônicos com neurolépticos e suas interações com disseleneto de difenila e *V. officinalis* sobre parâmetros de estresse oxidativo em fígado e rim de ratos.

Objetivos Específicos

1- Avaliar o efeito de tratamentos com neurolépticos e suas interações com disseleneto de difenila e *V. officinalis* sobre a atividade da enzima δ-ALA-D em fígado e rim de ratos;

2- Investigar o efeito de tratamentos com neurolépticos e suas interações com disseleneto de difenila e *V. officinalis* sobre parâmetros de estresse oxidativo e o status antioxidante em fígado e rim de ratos;

3- Verificar a atividade das enzimas aspartato aminotransferase (AST) e alanina aminotransferase (ALT) no soro de ratos tratados com haloperidol, *V. officinalis* ou a associação dos dois compostos.

3. ARTIGOS CIENTÍFICOS

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de artigos científicos, os quais se encontram aqui organizados. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se nos próprios artigos. O **artigo 1** e o **artigo 2** estão dispostos na forma em que foram submetidos para publicação.

3.1. – O TRATAMENTO CRÔNICO COM FLUFENAZINA ALTERA PARÂMETROS DE ESTRESSE OXIDATIVO EM FÍGADO E RIM DE RATOS

Artigo 1

CHRONIC TREATMENT WITH FLUPHENAZINE ALTERS PARAMETERS OF OXIDATIVE STRESS IN LIVER AND KIDNEY OF RATS

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Chronic treatment with fluphenazine alters parameters of oxidative stress in liver and kidney of rats

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Abstract

The aim of this study was to assess the toxic effects of chronic exposure to fluphenazine in liver and kidney of rats, as well as the protective effect of diphenyl diselenide on the fluphenazine-induced damage. Treatment with fluphenazine caused an increase in lipid peroxidation levels in liver and kidney homogenates, a decrease in hepatic SOD activity, and an increase in hepatic CAT activity. Diphenyl diselenide was able to protect liver and kidney from lipid peroxidation, ameliorate SOD activity in liver, and prevent the increase in hepatic CAT activity. Diphenyl diselenide treatment did not affect δ-aminolevulinate dehydratase (δ-ALA-D) activity, but fluphenazine alone or in combination with diphenyl diselenide showed an inhibitory effect on δ-ALA-D activity in liver and kidney. Diphenyl diselenide and fluphenazine treatment increased the reactivation index of hepatic δ-ALA-D. Taken together, these results indicate a relationship between the oxidative stress and fluphenazine treatment in liver and kidney of rats.

Key words: Fluphenazine; selenium; oxidative stress; δ-ALA-D; TBARS.

Introduction

Fluphenazine is one of the three antipsychotic drugs enlisted in the recent (14th) World Health Organization Model List of Essential Medicines (Ozdemir et al. 2006). However, the use of this first-generation antipsychotic medication can be associated with tardive dyskinesia (TD), a debilitating involuntary hyperkinetic movement disorder, in 20 – 50% of individuals with a psychotic illness during chronic treatment (Creese et al. 1976; Gunne et al. 1986; Ozdemir et al. 2006; See et al. 1992). Of particular importance, the use of fluphenazine and the symptoms of TD in humans or orofacial dyskinesia (OD) in rodents have been associated with oxidative stress (Abílio et al. 2004; Burger et al. 2003; Cadet et al. 1986; Lohr et al. 1988, 1990).

The use of phenothiazines like fluphenazine has been associated with hepatic injury (Ishak and Irey 1972; Jones et al. 1983; Regal et al. 1987). Indeed, agranulocytosis and the release of transaminase enzymes from liver cells are described as consequences of neuroleptic drug use (Munyon et al. 1987). Zimmerman (1968) reported elevations of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) on persons taking chlorpromazine, a phenothiazine derivative. The available evidence suggests that the release of AST and ALT can be due to the direct cytotoxic effect of phenothiazines on liver cells (Dujovne and Zimmerman 1969). Isolated elevations of hepatic enzymes occur frequently with phenothiazine drugs (frequency evaluated to 20%) (Dumortier et al. 2002). In this vein, literature has

indicated that phenothiazine causes cytotoxicity in hepatocytes, which can be prevented by the antioxidants (Eghbal et al. 2004).

Seleno-organic compounds have been studied based on their potential antioxidant properties (Mugesh et al. 2001; Rayman 2000). In fact, this class of compounds exhibits glutathione peroxidase-like activity and oxidizes sulfhydryl groups (-SH) during the reduction of H_2O_2 (Klotz et al. 2003; Muller et al. 1985; Nogueira et al. 2002; Wendel et al. 1984). Of particular importance, ebselen has antioxidant properties in a variety of in vitro and in vivo models of neurotoxicity in rats (Imai et al. 2001; Moussaoui et al. 2000; Namura et al. 2001; Porciúncula et al. 2001; Rossato et al. 2002). Recent data from our laboratory indicated that ebselen plays a protective role against reserpine-induced OD and reverses the increase in thiobarbituric acid-reactive species (TBARS) production caused by reserpine administration (Burger et al. 2003). Ebselen has also been demonstrated to protect liver when injury was induced by paracetamol, CCl_4, lipopolysaccharide and *Propionibacterium acnes*, alcohol, and ischemia-reperfusion injury (Kono et al. 2001; Koyanagi et al. 2001; Li et al. 1994; Ozaki et al. 1997; Wasser et al. 2001).

The simplest of diaryl diselenides, diphenyl diselenide, has been shown to be even more active as a glutathione peroxidase mimic (Wilson et al. 1989) and less toxic to rodents than ebselen (Meotti et al. 2003; Nogueira at al. 2003). Recently, data from our laboratory have indicated that diphenyl diselenide decreased the prevalence of vacuous chewing movements induced by long-

term treatment with fluphenazine in rats (Fachinetto et al. 2007). Furthermore, diphenyl diselenide has a protective role in a variety of experimental models associated with the overproduction of free radicals in the brain, liver, and kidney (Borges et al. 2005, 2006; Burger et al. 2004; Ghisleni et al. 2003; Rossato et al. 2002; Santos et al. 2005a). In contrast, several researches have demonstrated that liver is a target of selenorganic compound actions, as well as the various clinical conditions in which hydroperoxides play a role (Nogueira et al. 2004).

δ-Aminolevulinate dehydratase (δ-ALA-D) is a sulfhydryl-containing enzyme highly susceptible to oxidizing agents and is inhibited in different pro-oxidant situations (Barbosa et al. 1998; Farina et al. 2002; Folmer et al. 2002, 2003; Gonçalves et al. 2005; Santos et al. 2005b; Soares et al. 2003). The inhibition of δ-ALA-D may impair the heme biosynthesis and may result in the accumulation of aminolevulinic acid (ALA) that has been demonstrated to be a pro-oxidant molecule under significant physiological conditions (Bechara et al. 1993; Bechara 1996; Emanuelli et al. 2001). Based on this, δ-ALA-D can be suggested as a marker of oxidative stress.

The hepatotoxicity mechanism of phenothiazines is not completely understood but may involve a combination of physiochemical, immuno-allergic, and oxidative stress induced toxicity (Dumortier 2002; Eghbal et al. 2004; Regal 1987). In spite of the several reports of hepatic injury of phenothiazinic drugs, few data are available about their effects on kidney or whether oxidative stress

could be involved on these effects. In this way, the rationale for this study was to evaluate the oxidative stress in liver and kidney of rats chronically treated with fluphenazine, a phenothiazine, as well as to assess the potential protective effect of diphenyl diselenide on the fluphenazine-induced damage.

Materials and Methods

Chemicals

Fluphenazine enantate (Flufenan®) was kindly donated by Cristália (Brazil). Diphenyl diselenide was synthesized by the method previously described (Paulmier 1986). Thiobarbituric acid, aminolevulinic acid, and DL-dithiothreitol (DTT) were obtained from Sigma (St. Louis, MO, USA). $HgCl_2$, NaCl, K_2HPO_4, KH_2PO_4, trichloroacetic acid (TCA), *para*-dimethylaminobenzaldehyde, and glacial acetic acid were purchased from Reagen (Rio de Janeiro, RJ, Brazil). All other chemicals were purchased from Merck (Darmstadt, Germany).

Animals

Male Wistar rats weighing 270–320 g and with age from 3 to 3.5 months from our own breeding colony were kept in cages of three or four animals each. They were placed in a room with controlled temperature (22±3°C) on a 12-h light/dark cycle with lights on at 7:00 A.M, and had continuous access to food and water. The animals were maintained and used in accordance to the guidelines of the Brazilian Association for Laboratory Animal Science.

Treatment

For chronic treatment, rats were divided into control, diphenyl diselenide, fluphenazine, and fluphenazine plus diphenyl diselenide groups. Fluphenazine enantate was administered intramuscularly (i.m.) every 21 days (25 mg/kg, i.m.). Diphenyl diselenide was dissolved in soy oil and administered subcutaneously (s.c.) three times per week in nonconsecutive days (1 mg/kg, s.c.). The control group received soy oil (1 mL/kg) in the same way as the diphenyl diselenide group. The treatment was carried out over the course of 6 months and was based on previous studies (See et al. 1992; Van Kampen and Stoessl 2000; Burger et al. 2006; Fachinetto et al. 2007).

Animals were divided into four groups of 9 animals each:

Control: received soy oil (1mL/kg) every 21 days (i.m.) and 3 times a week in alternating days (s.c.);

Diphenyl diselenide: received diphenyl diselenide 3 times a week in alternating days (1mg/kg, s.c.), and the vehicle (1mL/kg soy oil) was administered at each 21 days (i.m.);

Fluphenazine: received fluphenazine enantate at each 21 days (25 mg/kg, i.m.), and the vehicle (1mL/kg soy oil) was administered 3 times a week in alternating days (s.c.);

Combined treatment: received fluphenazine enantate at each 21 days (25 mg/kg, i.m.), and diphenyl diselenide 3 times a week in alternating days (1mg/kg, s.c.).

Tissue preparation

Animals were killed by decapitation. Liver and kidney were quickly removed, placed on ice, and homogenized at 7 and 5 volumes of 0.9% NaCl, respectively. The homogenates were centrifuged at 4,000 x g for 10 min to yield a low-speed supernatant fraction (S1) that was used for the biochemical and enzymatic assays. In order to perform SOD and CAT assays, S1 was diluted as described in the respective sections.

Lipid peroxidation assay

Thiobarbituric acid reactive species (TBARS) were determined as described by Ohkawa et al. (1979). In brief, samples were incubated at 100 ºC for 1 h in a medium containing 8.1 % sodium dodecyl sulfate, 1.4 M acetic acid, pH 3.4, and 0.6% thiobarbituric acid. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde (MDA), a secondary product of lipid peroxidation, was measured spectrophotometrically at 532 nm. Results were expressed as nmol of MDA/ gram of tissue.

Enzyme assay

δ-ALA-D activity

δ-ALA-D activity was assayed according to the method of Sassa (1982) by measuring the rate of product (porphobilinogen/PBG) formation. The reaction product was determined using modified Ehrlich's reagent at 555 nm with a molar absorption coefficient of 6.1×10^{4} M^{-1} for the Ehrlich-PBG salt. The incubation medium contained δ-ALA 2.4 mM and potassium phosphate buffer

(pH 6.8) 0.084 M. The reaction was initiated by the addition of enzymatic material and the incubations were carried out for 90 and 150 minutes, for liver and kidney respectively, at 39°C. Afterwards, the reaction was stopped by the addition of TCA 10% containing $HgCl_2$ 0.01 M. The activity of δ-ALA-D was expressed as nmol of PBG/ mg of protein/ h. Simultaneously, a set of tubes was assayed using the same protocol, except that 2 mM DTT was added in order to obtain the reactivation index. This index indicates the extent of the reactivation of δ-ALA-D activity. The reactivation index of δ-ALA-D activity was calculated as follows:

$$\frac{(\text{δ-ALA-D activity with DTT} - \text{δ-ALA-D activity without DTT})}{\text{δ-ALA-D activity with DTT}} \times 100\%$$

SOD activity

To verify SOD activity, S1 of kidney and liver were adequately diluted to 40 and 60 volumes with 0.9% NaCl, respectively, and the assay was performed according to the method of Misra and Fridovich (1972). Briefly, epinephrine rapidly autooxidizes at pH 10.5 producing adrenochrome, a pink-colored product that can be detected at 480 nm. The addition of samples (10, 20, 30 µL) containing SOD inhibits the autooxidation of epinephrine. The rate of inhibition was monitored during 180 seconds at intervals of 30 seconds. The amount of enzyme required to produce 50% inhibition at 25°C was defined as one unit of enzyme activity (UI).

CAT activity

CAT activity was measured by the method of Aebi (1974). An aliquot of liver and kidney supernatants (10 µL) diluted with 60 and 40 volumes of 0.9% NaCl, respectively, was added to a quartz cuvette and the reaction was started by the addition of freshly prepared H_2O_2 (30 mM) in phosphate buffer (50 mM, pH 7). The rate of H_2O_2 decomposition was measured spectrophotometrically at 240 nm during 120 seconds at intervals of 15 seconds. CAT activity was expressed as percentage of control.

Protein measurement

Protein was assayed by the method of Lowry et al. (1951) with serum bovine albumin as standard.

Statistical analysis

Data were analyzed statistically by one-way ANOVA, followed by Duncan's post-hoc tests. The results were considered statistically significant when $p<0.05$.

Results

Lipid Peroxidation

Chronic treatment with fluphenazine increased TBARS production in the liver when compared to control and diphenyl diselenide groups ($p<0.05$). Diphenyl diselenide administration did not modify hepatic TBARS levels. However, in the combined treatment, diphenyl diselenide caused a decrease in

hepatic TBARS levels observed after fluphenazine treatment, returning TBARS levels to control values (Fig. 1A).

Long-term treatment with fluphenazine caused an increase in TBARS levels of about 50% in kidney homogenates when compared to the control group ($p<0.05$). Treatment with diphenyl diselenide did not change renal TBARS levels, whereas when the combined treatment was used, diphenyl diselenide reduced renal TBARS enhanced by fluphenazine to control level (Fig. 1B).

δ-ALA-D activity

Diphenyl diselenide treatment did not change hepatic δ-ALA-D activity (Fig. 2A). However, fluphenazine inhibited δ-ALA-D activity in liver (Fig. 2A, $p<0.05$) and the combined treatment did not restore the enzyme activity. In vitro, DTT, a classical agent that restores oxidized thiol groups, caused an increase in hepatic δ-ALA-D activity of all groups (data not show). In fact, the combined treatment increased the hepatic δ-ALA-D reactivation index and this increase was the highest among the groups (Fig. 2B, $p<0.05$).

In kidney, fluphenazine alone did not alter δ-ALA-D activity (Fig. 3A). However, when the combined treatment was used, the δ-ALA-D activity decreased (Fig. 3A, $p<0.05$). In vitro, DTT restored δ-ALA-D activity in kidney homogenates (data not show) as well as in the liver. Renal δ-ALA-D reactivation index values were not modified by diphenyl diselenide, fluphenazine or combined treatment (Fig. 3B).

SOD activity

Long-term treatment with fluphenazine caused a significant decrease (about 50%) in hepatic SOD activity ($p<0.05$). Treatment with diphenyl diselenide did not modify hepatic SOD activity. However, in the combined treatment, organoselenium compound recovered hepatic SOD activity inhibited by fluphenazine. In fact, the activity of the combined treatment was not significantly different from control or diphenyl diselenide alone groups (Fig. 4A). Isolated treatment with either diphenyl diselenide or fluphenazine did not change renal SOD activity. However, the combined treatment caused a reduction in renal SOD activity (Fig. 4B, $p<0.05$).

CAT activity

Treatment with fluphenazine caused a significant increase in CAT activity of rat liver homogenates ($p<0.05$). Isolated treatment with diphenyl diselenide did not change hepatic CAT activity. However, combined treatment partially prevented the increase in CAT activity caused by fluphenazine (Fig. 5A). Renal catalase activity was not modified by diphenyl diselenide, fluphenazine or the combined treatment (Fig. 5B).

Discussion

The present investigation was carried out with the purpose to evaluate oxidative stress in liver and kidney of rats chronically treated with fluphenazine. We demonstrated here that the long-term treatment with fluphenazine caused an increase in lipid peroxidation (TBARS), a reduction in SOD activity, and an

increase in CAT activity in liver, showing a relationship of fluphenazine administration and oxidative stress. Fluphenazine also induced lipid peroxidation in kidney, although CAT and SOD activities were not altered in this organ.

Phenothiazines are extensively oxidized in the body to form cation radicals (Yang and Kulkarni 1997), which are believed to be sulfur centered cation radicals as sulfoxide was the end product (Cheng et al. 1978; Hammerich and Parker 1983). It has been hypothesized that peroxidase-catalyzed drug oxidation causes protein binding and oxidative stress, which can contribute with cell death (Tafazoli and O'Brien 2005). Furthermore, hepatocyte cytotoxicity induced by phenothiazines was markedly enhanced by nontoxic concentrations of extracellular H_2O_2/peroxidase, and also caused ascorbate, GSH and NADH cooxidation and reactive oxygen species formation (Eghbal et al. 2004). In this way, we could suggest that the fluphenazine-induced lipid peroxidation could result from the production of fluphenazine radical metabolites catalyzed by peroxidases.

Fluphenazine chronic treatment induced alterations in hepatic SOD and CAT enzymes activities. These findings are consistent with those of Cadet and Perumal (1990), who reported alterations of SOD and CAT activity in the brain of rats after chronic fluphenazine treatment. Other researchers have been documented reduced SOD activity in rat brain treated with haloperidol which probably was the result of alterations in genetic expression of these enzymes

(Parikh et al. 2003). The decrease in hepatic SOD activity caused by fluphenazine treatment also could contribute to the increase in TBARS levels in the liver.

Diphenyl diselenide was used in this study based on its hepatoprotective and antioxidant properties (Borges et al. 2005, 2006). Indeed, diphenyl diselenide was effective in protecting liver and kidney against lipid peroxidation induced by fluphenazine. This protective effect on TBARS was accompanied by a partial restoration of CAT activity in liver. Diphenyl diselenide was able to ameliorate SOD activity in liver of rats treated with fluphenazine. In this way, the protective effects of diphenyl diselenide could be attributed to the thiol peroxidase-like activity that has been described for organoselenium compounds and to other antioxidant properties of diphenyl diselenide (Arteel et al. 2001; Nogueira et al. 2004; Rossato et al. 2002). On the other hand, the renal SOD activity was diminished by the combined treatment. The decrease in SOD activity in this group was unexpected and may indicate a complex interaction between the antioxidant properties of the selenium compound and a decrease in an important antioxidant enzyme in kidney. Nevertheless, the administration of diphenyl diselenide was not accompanied by any sign of lipid peroxidation (TBARS) in the kidney.

Fluphenazine treatment caused an inhibition of hepatic δ-ALA-D activity, and the combination of fluphenazine and diphenyl diselenide was unable to restore the enzyme activity. In fact, this combination increased the partial

inhibition caused by fluphenazine alone. Diphenyl diselenide can oxidize the cysteinyl residues in the active site of δ-ALA-D maybe due to a thiol nucleophilic attack in the selenium atom of diphenyl diselenide to give an unstable intermediate of the type E-Cys-S-SePh and selenophenol. Subsequently, the other cysteinyl residue attacks the sulfur-selenium bound of the intermediate producing the oxidized enzyme and regenerates a second molecule of selenophenol (Barbosa et al. 1998; Farina et al. 2002). However, in this case diphenyl diselenide alone did not affect δ-ALA-D activity, only when associated to fluphenazine. In this way, the interaction of fluphenazine and diphenyl diselenide could provoke the oxidation of δ-ALA-D sulfhydryl groups in a more pronounced way than fluphenazine alone. This is supported by the fact that DTT, a reducing agent that restores oxidized thiol containing enzymes (Perottoni et al. 2005) could restore the inhibition of δ-ALA-D activity, and by the reactivation index, which was higher in rats treated with the combined treatment than other treated groups.

Quite the opposite of liver, fluphenazine alone did not cause any effect on renal δ-ALA-D activity, although the combination of fluphenazine and diphenyl diselenide resulted in inhibition of δ-ALA-D activity. DTT was able to restore δ-ALA-D activity in kidney. However, we did not observed differences in the reactivation index for δ-ALA-D among the groups. In this way, we could suggest that the mechanism underlying the inhibitory effect of these compounds on renal δ-ALA-D was not related to the oxidation of -SH groups. The

decreased activity of the renal δ-ALA-D in the combined treatment could be attributed to an additive effect of fluphenazine and diphenyl diselenide on the enzyme activity.

The treatment carried out in this work was based on previous studies and is a rat model of TD (Fachinetto et al. 2007; See et al. 1992; Van Kampen and Stoessl 2000). Several authors presented evidence of the involvement of reactive oxygen species in the development of TD (Abílio et al. 2004; Burger et al. 2003; Cadet et al. 1986; Lohr et al. 1988). In this way, the results presented here could corroborate this hypothesis. The same cause of hepatic oxidative stress may also trigger brain oxidative stress. In fact, one can suppose that even a limited hepatotoxicity of neuroleptics might facilitate their neurotoxicity, particularly, by increasing the susceptibility of the entire organism to the damaging effect of free radicals.

Drug-induced liver toxicity is common and accounts for approximately one-half of the cases of acute liver failure (Kaplowitz 2001). Neuroleptic drugs have been implicated in biological or/and clinical hepatotoxicity although the precise mechanisms remain unclear (Dumortier 2002). Since antipsychotics are going to be the drugs of choice for the treatment of psychotic disorders, the understanding of the effects of their action on oxidative stress and oxidative cellular injury may be very important (Parikh et al. 2003). This study demonstrated for the first time an association between oxidative stress and fluphenazine chronic treatment in liver and kidney of rats. Moreover, these data

may provide useful indications about the benefits of diphenyl diselenide administration to protect liver from a variety of hepatotoxicants since diphenyl diselenide protected from oxidative damage caused by fluphenazine in liver of rats. However, we believe that further studies are necessary to test the hypothesis of whether oxidative stress could contribute to the fluphenazine-induced hepatotoxicity.

References

Abílio V C, Silva R H, Carvalho R C, Grassl C, Calzavara M B, Registro S, D'Almeida V, Ribeiro R A, Frussa-Filho R (2004) Important role of striatal catalase in aging- and reserpine-induced oral dyskinesia. Neuropharmacology 47:263-272

Aebi H (1974) Catalase. In: Bergmeyer H V (ed) Methods of Enzymatic Analysis, VEB Chemie Weinheim, pp. 673-677

Arteel G E, Sies H (2001) The biochemistry of selenium and glutathione system. Environ Toxicol Pharmacol 10:153-158

Barbosa N B V, Rocha J B T, Zeni G, Emanuelli T, Beque M C, Braga A L (1998) Effect of organic forms of selenium on (-aminolevulinate dehydratase from liver, kidney and brain of adult rats. Toxicol Appl Pharmacol 49:243-253

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

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

Borges L P, Borges V C, Moro A V, Nogueira C W, Rocha J B T, Zeni G (2005) Protective effect of diphenyl diselenide on acute liver damage induced by 2-nitropropane in rats. Toxicology 210:1–8

Borges L P, Nogueira C W, Panatieri R B, Rocha J B T, Zeni G (2006) Acute liver damage induced by 2-nitropropane in rats: Effect of diphenyl diselenide on antioxidant defenses. Chem Biol Interact 160:99–107

Burger M E, Alves A, Callegari L, Athayde F R, Nogueira C W, Zeni G, Rocha J B T (2003) Ebselen attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum. Prog Neuro-psychopharmacol 27:135-140

Burger M E, Fachinetto R, Calegari L, Paixão M W, Braga A L, Rocha J B T (2004) Effects of age on orofacial dyskinesia reserpine-induced and possible protection of diphenyl-diselenide. Brain Res Bull 64:339-345

Burger M B, Fachinetto R, Wagner C, Perottoni J, Pereira R P, Zeni G, Rocha J B T (2006) Effects of diphenyl diselenide on orofacial dyskinesia model in rats. Brain Res Bull 70:165–170

Cadet J L, Lohr J B, Jeste D V (1986) Free radical and tardive dyskinesia. Trends Neurosci 9:107-108

Cadet J L, Perumal A S (1990) Chronic treatment with prolixine causes oxidative stress in rat brain. Biol Psych 28:738–740

Cheng H Y, Sackett P H, McCreeny R L (1978) Reactions of chlorpromazine cation radical with physiologically occurring nucleophiles. J Med Chem 21:948–952

Creese I, Burt D R, Snyder S H (1976) Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science 192:481-483

Dujovne C A, Zimmerman H T (1969) Cytotoxicity of phenothiazines on Chang liver cells as measured by enzyme leakage. Proc Soc Exp Biol Med 131:583-587

Dumortier G, Cabaret W, Stamatiadis L, Saba G, Benadhira R, Rocamora J F, Aubriot-Delmas B, Glikman J, Januel D (2002) Hepatic tolerance of atypical antipsychotic drugs. Encephale 28:542-551

Eghbal M A, Tafazoli S, Pennefather P, O'Brien P J (2004) Peroxidase catalysed formation of cytotoxic prooxidant phenothiazine free radicals at physiological pH. Chem Biol Interact 151:43-51

Emanuelli T, Pagel F W, Alves L B, Regner A, Souza D O (2001) Inhibition of adenylate cyclase activity by 5-aminolevulinic acid in rat and human brain. Neurochem Int 38:213-218

Fachinetto R, Villarinho J G, Wagner C, Pereira R P, Puntel R L, Paixão M W, Braga A L, Burger M E, Calixto J B, Rocha J B, Ferreira J (2007) Diphenyl diselenide decreases the prevalence of vacuous chewing movements induced by fluphenazine in rats. Psychopharmacology (Berl) 194:423-432

Farina M, Barbosa N B V, Nogueira C W, Folmer V, Zeni G, Andrade L H, Braga A L, Rocha J B T (2002) Reaction of diphenyl diselenide with hydrogen peroxide and inhibition of delta-aminolevulinate dehydratase from rat liver and cucumber leaves. Braz J Med Biol Res 35:623-631

Folmer V, Soares J C, Rocha J B T (2002) Oxidative stress in mice is dependent on the free glucose content of the diet. Inter J Biochem Cell Biol 34:1279-1285

Folmer V, Soares J C, Gabriel D, Rocha J B T (2003) A high fat diet inhibits delta-aminolevulinate dehydratase and increases lipid peroxidation in mice (Mus musculus). J Nutr 133:2165-2170

Ghisleni G, Porciúncula L, Cimarosti H, Rocha J B T, Salbego C S, Souza D O (2003) Diphenil diselenide protects rat hippocampal slices submitted to oxygen–glucose deprivation and diminishes inducible nitric oxide synthase immunocontent. Brain Res 986:196–199

Gonçalves T L, Erthal F, Corte C L D, Muller L G, Piovezan C M, Nogueira C W, Rocha J B T (2005) Involvement of oxidative stress in the pre-malignant and malignant states of cervical cancer in women. Clin Biochem 38:1071-1075

Gunne L M, Andersson U, Bondesson U, Johansson P (1986) Spontaneous chewing movements in rats during acute and chronic antipsychotic drug administration. Pharmacol, Biochem, Behav 25:897–901

Hammerich O, Parker V D (1983) The kinetics and mechanisms of the reactions of cation radicals of phenothiazine-derivatives with acetate ion and water in acetonitrile. Acta Chem Scand. B: Org Chem Biochem 37**:**303–311

Imai H, Masayasu H, Dewar D, Graham D I, Macrae I M (2001) Ebselen protects both gray model of focal and white matter in a rodent cerebral ischemia. Stroke 32:2149–2156

Ishak K G, Irey N S (1972) Hepatic injury associated with the phenothiazines. Arch Pathol 93:283-304

Jones J K, Van de Carr S W, Zimmerman H, Leroy A (1983) Hepatotoxicity associated with phenothiazines. Psychopharmacol Bull 19:24-27

Kaplowitz N (2001) Drug-induced liver disorders: implications for drug development and regulation. Drug Saf. 24:483–490

Klotz L O, Sies H (2003) Defenses against peroxynitrite: selenocompounds and flavonoids. Toxicol Lett 14:125–132

Kono H, Arteel G E, Rusyn I, Sies H, Thurman R G (2001) Ebselen prevents early alcohol-induced liver injury in rats. Free Radic Biol Med 30:403-411

Koyanagi T, Nakamuta M, Enjoji M, Iwamoto H, Motomura K, Sakai H, Nawata H (2001) The selenoorganic compound ebselen suppresses liver injury induced by *Propionibacterium acnes* and lipopolysaccharide in rats. Int J Mol Med 7:321-327

Lee W L (2003) Drug-Induced Hepatotoxicity. New Eng J Med 349:474-485

Li Q -J, Bessems J G, Commandeur J N, Adams B, Vermeulen N P (1994) Mechanism of protection of ebselen against paracetamol-induced toxicity in rat hepatocytes. Biochem Pharmacol 48:1631-1640

Lohr J B, Cadet J L, Lohr M A, Larson L, Wasli E, Wade L (1988) Vitamin E in the treatment of tardive dyskinesia: The possible involvement of free radical mechanisms. Schizophr Bull 14:291-296

Lohr J B, Kuczenski R, Bracha H S, Moir M, Jeste D V (1990) Increased indices of free radical activity in the cerebrospinal fluid of patients with tardive dyskinesia. Biol Psychiatry 28:535-539

Lowry O H, Rosebrough N J, Farr A L, Randall R J (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275

Meotti F C, Borges V C, Zeni G, Rocha J B T, Nogueira C W (2003) Potential renal and hepatic toxicity of diphenyl diselenide, diphenyl ditelluride and ebselen for rats and mice. Toxicol Lett 143:9–16

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

Moussaoui S, Obinu M, Daniel N, Reibaud M, Blanchard V, Imperato A (2000) The antioxidant ebselen prevents neurotoxicity and clinical symptoms in a primate model of Parkinson’s disease. Exp Neurol 166:235–245

Mugesh G, du Mont W W, Sies H (2001) Chemistry of biologically important synthetic organoselenium compounds. Chem Rev 1001:2125–2179

Müller A, Gabriel H, Sies H (1985) A novel biologically active selenoorganic compound protective glutathione-dependent effect of PZ 51 (Ebselen) against ADP-Fe-induced lipid peroxidation in isolated hepatocytes. Biochem Pharmacol 34:1185-1189

Munyon W H, Salo R, Briones D F (1987) Cytotoxic effects of neuroleptic drugs. Psychopharmacology (Berl) 91:182-188

Namura S, Nagata I, Takami S, Masayasu H, Kikuchi H (2001) Ebselen reduces cytochrome c release from mitochondria and subsequent DNA fragmentation after transient focal cerebral ischemia in mice. Stroke 32:1906–1911

Nogueira C W, Rotta L N, Zeni G, Souza D O, Rocha J B T (2002) Exposure to ebselen changes glutamate uptake and release by rat brain synaptosomes. Neurochem Res 27:283–288

Nogueira C W, Meotti F C, Curte E, Pilissão C, Zeni G, Rocha J B T (2003) Investigations into the potential neurotoxicity induced by diselenides in mice and rats. Toxicology 183:29-37

Nogueira C W, Zeni G, Rocha J B T (2004) Organoselenium and organotellurium compounds: Toxicology Pharmacology. Chem Rev 104:6255-6285

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

Ozaki M, Nakamura M, Teraoka S, Ota K (1997) Ebselen, a novel anti-oxidant compound, protects the rat liver from ischemia-reperfusion injury. Transpl Int 10:96-102

Ozdemir V, Aklillu E, Mee S, Bertilsson L, Graham J E, Caligiuri M, Lohr J B, Reist C (2006) Pharmacogenetics for off-patent antipsychotics: reframing the risk for tardive dyskinesia and access to essential medicines. Expert Opin Pharmacother 7:119-133

Parikh V, Khan M M, Mahadik S P (2003) Differential effects of antipsychotics on expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. J Psych Res 37:43–51

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

Perottoni J, Meotti F C, Folmer V, Pivetta L, Nogueira C W, Zeni G, Rocha J B T (2005) Ebselen and diphenyl diselenide do not change the inhibitory effect of lead acetate on delta aminolevulinate dehydratase. Environ Toxicol Pharmacol 19:239-248

Porciúncula L O, Rocha J B T, Boeck C R, Vendite D, Souza D O (2001) Ebselen prevents excitotoxicity provoked by glutamate in rat cerebellar granule neurons. Neurosci Lett 299:217–220

Rayman M P (2000) The importance of selenium to human health. Lancet 356:233–241

Regal R E, Billi J E, Glazer H M (1987) Phenothiazine-induced cholestatic jaundice. Clin Pharm 10:787-794

Rossato J I, Ketzer L A, Centurião F B, Silva S J N, Lûdtke D S, Zeni G, Braga A L, Rubin M A, Rocha J B T (2002) Antioxidant properties of new chalcogenides against lipid peroxidation in rat brain. Neurochem Res 27:297–303

Santos F W, Zeni G, Rocha J B T, Weis S N, Fachinetto J M, Favero A M, Nogueira C W (2005) Diphenyl diselenide reverses cadmium-induced oxidative damage on mice tissues. Chem Biol Interac 151:159–165a

Santos F W, Zeni G, Rocha J B T, Nascimento P C, Marques M S, Nogueira C W (2005) Efficacy of 2,3-dimercapto-1-propanesulfonic acid (DMPS) and diphenyl diselenide on cadmium induced testicular damage in mice. Food Chem Toxicol 43:1723-1730b

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

See R E, Chapman M A, Klitenick M A (1992) Chronic neuroleptic administration decreases extracellular GABA in the nucleus accumbens but not in the caudate-putamen of rats. Brain Res 588:177-180

Soares J C, Folmer V, Rocha J B T (2003) Influence of dietary selenium supplementation and exercise on thiol-containing enzymes in mice. Nutrition 19:627-632

Tafazoli S, O'Brien P J (2005) Peroxidases: a role in the metabolism and side effects of drugs. Drug Discov Today 10:617-625

Van Kampen J M, Stoessl A J (2000) Dopamine D_{1A} receptor function in a rodent model of tardive dyskinesia. Neuroscience 101:629-635

Wasser S, Lim G Y, Ong C N, Tan C E (2001) Anti-oxidant ebselen causes the resolution of experimentally induced hepatic fibrosis in rats. J Gastroenterol Hepatol 16:1244-1253

Wendel A, Fausel M, Safayhi H, Tiegs G, Otter R (1984) A novel biologically active seleno-organic compound-II. Activity of PZ 51 in relation to glutathione peroxidase. Biochem Pharmacol 33:3241–3245

Wilson S R, Zucker P A, Huang R -R C, Spector A (1989) A development of synthetic compounds with glutathione peroxidase activity. J Am Chem Soc 111:5936–5939

Yang X, Kulkarni A P (1997) Oxidation of phenothiazines by human term placental peroxidase in non-smokers. Teratog. Carcinog, Mutagen 17:139-151

Zimmerman H J (1968) Spectrum of hepatotoxicity. Perspect Biol Med 12:135-161

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Legends

Figure 1. Effect of diphenyl diselenide and/or fluphenazine treatments on TBARS production in liver (A) and kidney (B) homogenates. Data are expressed as mean ± S.E.M. for nine rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 2. Effect of diphenyl diselenide and/or fluphenazine treatments on δ-ALA-D activity in liver homogenates (A) and on the enzyme reactivation index (B). Data are expressed as mean ± S.E.M. for nine rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 3. Effect of diphenyl diselenide and/or fluphenazine treatments on δ-ALA-D activity in kidney homogenates (A) and on the enzyme reactivation index (B). Data are expressed as mean ± S.E.M. for nine rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 4. Effect of diphenyl diselenide and/or fluphenazine treatments on SOD activity in liver (A) and kidney (B) homogenates. Data are expressed as mean ± S.E.M. for nine rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 5. Effect of diphenyl diselenide and/or fluphenazine treatments on CAT activity in liver (A) and kidney (B) homogenates. Data are expressed as mean ± S.E.M. for nine rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test. The CAT activity in control groups was 291.41 and 173.25 μmol H_2O_2/mg protein/min for liver and kidney enzyme, respectively.

Figure 1.

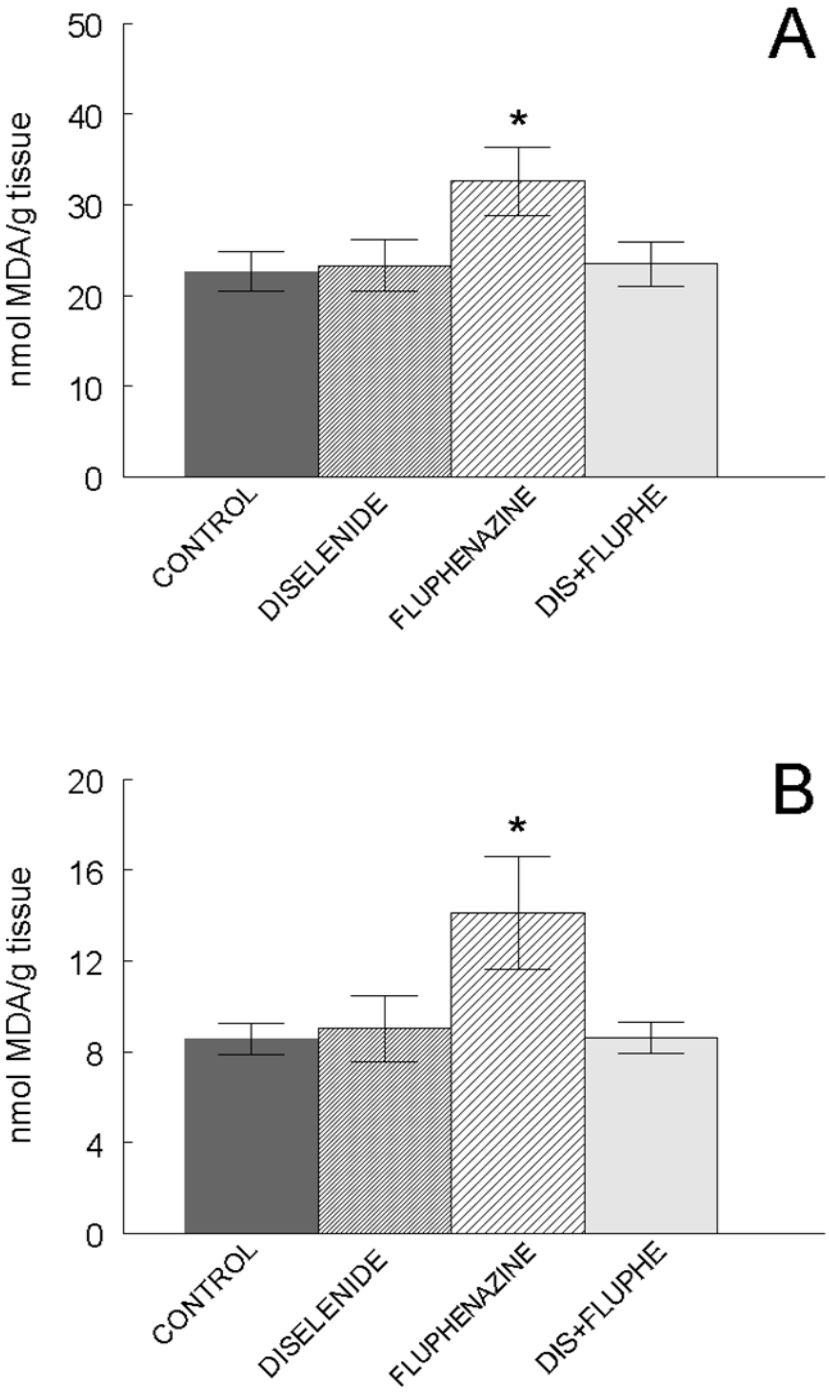
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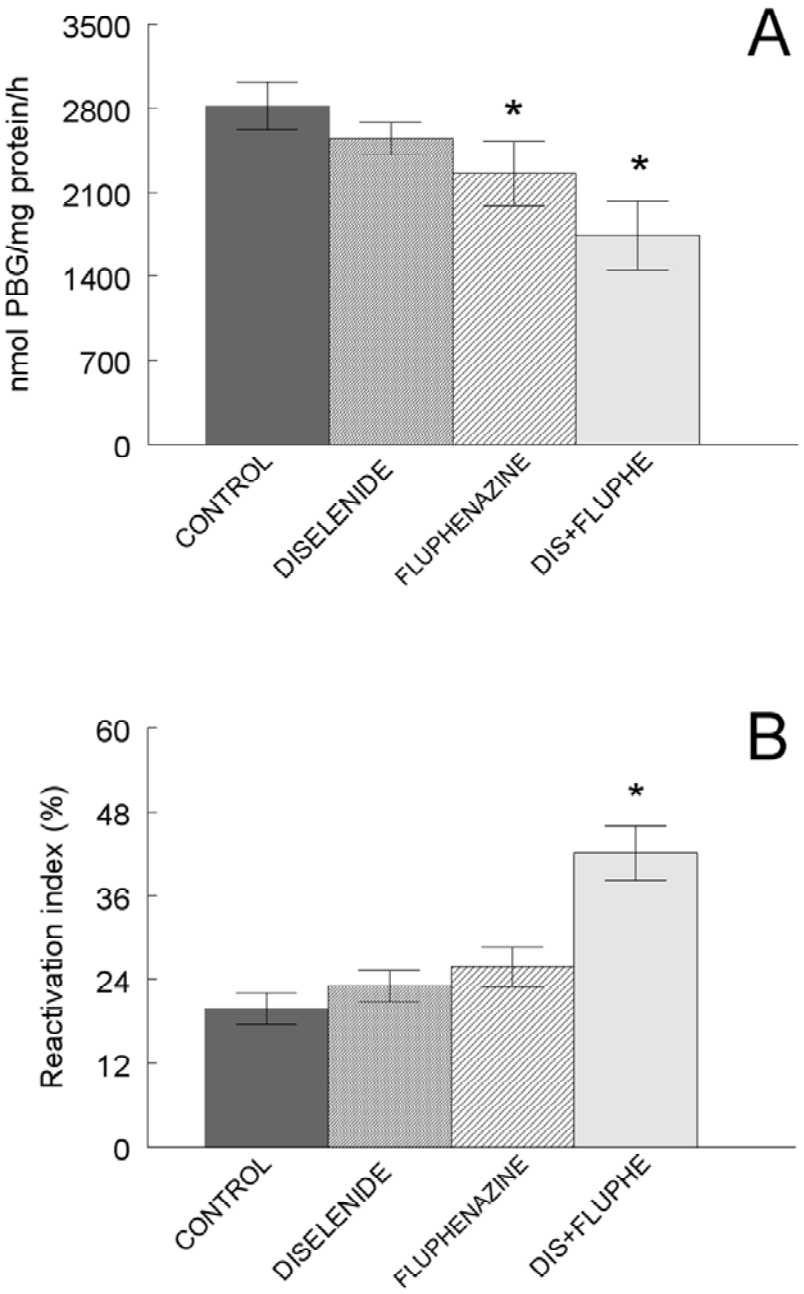
Figure 2.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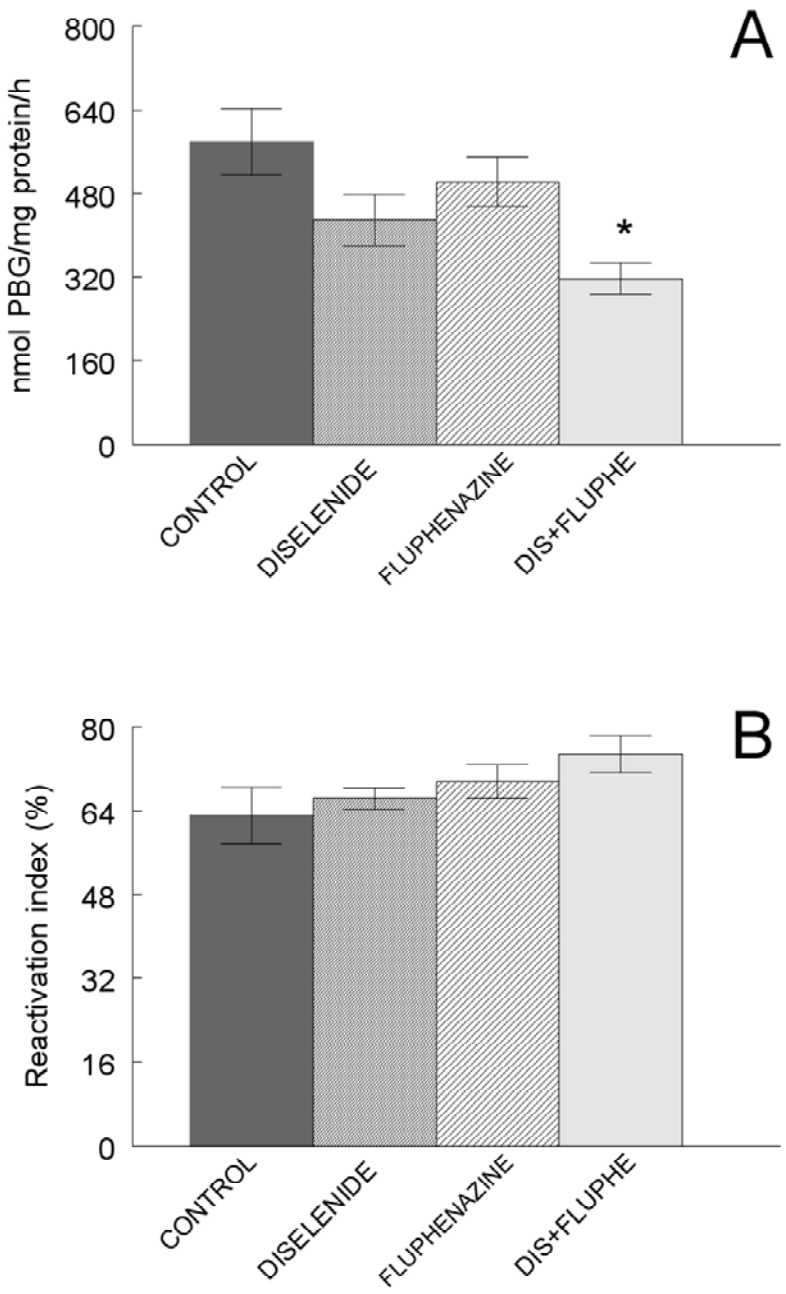
DIS+FLUPHE

Figure 3.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Reactivation index (%)
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Figure 4.

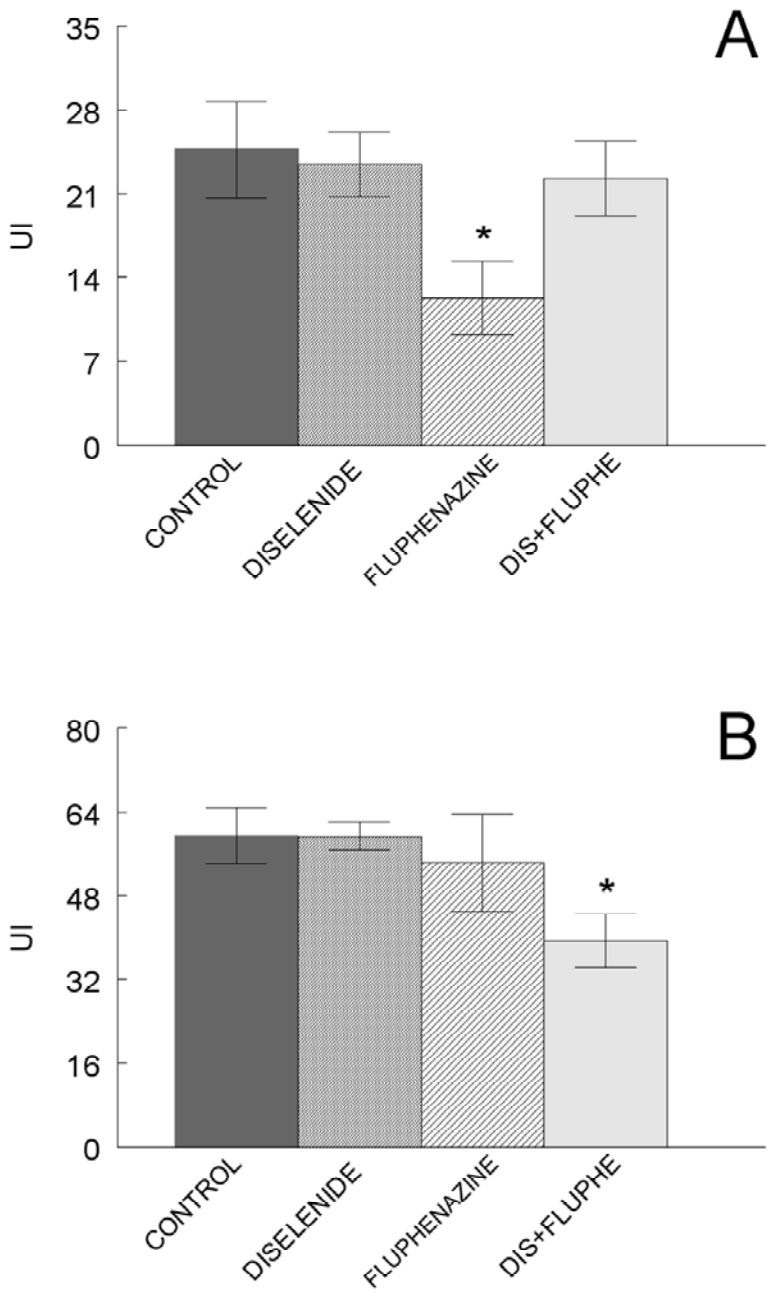
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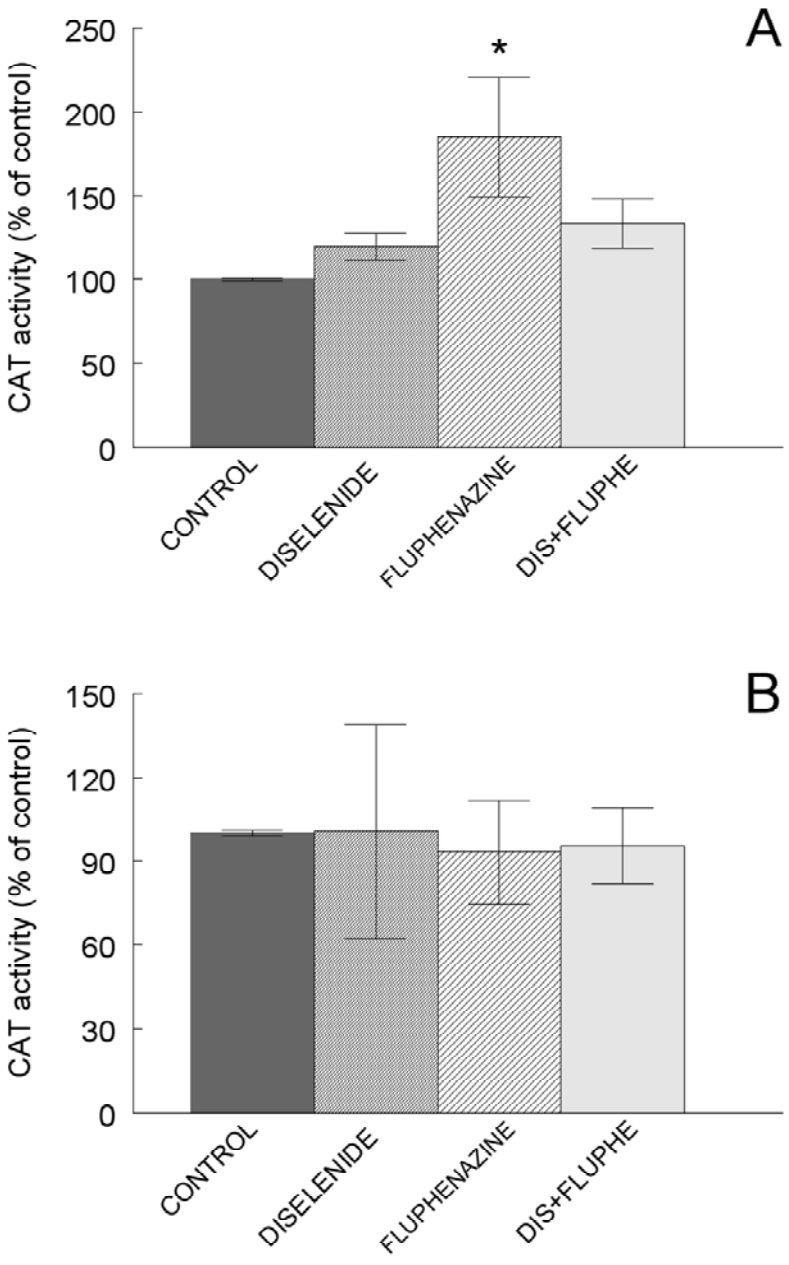
Figure 5.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CAT activity (% of control)
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CAT activity (% of control)
150
120
90
60
30
0
CONTROL
DISELENIDE
FLUPHENAZINE
DIS+FLUPHE

3.2 – INTERAÇÕERS POTENCIALMENTE ADVERSAS ENTRE HALOPERIDOL E VALERIANA

Artigo 2

POTENTIALLY ADVERSE INTERACTIONS BETWEEN HALOPERIDOL AND VALERIAN

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Potentially adverse interactions between haloperidol and valerian

Dalla Corte, C.L., Fachinetto, R., Colle, D., Pereira, R.P., Ávila, D.S., Villarinho, J.G., Wagner, C., Pereira, M.E., Nogueira, C.W., Soares, F.A.A.* and Rocha, J.B.T.

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Running title: Haloperidol, valerian and hepatic damage

Keywords: Haloperidol; *Valeriana officinalis*; Herb-drug interaction; Oxidative stress; δ-ALA-D.

Abstract

This study was designed to determine whether the treatment of haloperidol (HP), valerian or both in association impairs the liver or kidney functions. Valerian alone did not affect oxidative stress parameters in the liver or kidney of rats. HP alone only increased glutathione (GSH) depletion in liver, but not in kidney. However, when HP was associated with valerian, an increase in lipid peroxidation levels and dichlorofluorescein (DCFH) reactive species production was observed in the hepatic tissue. Superoxide dismutase (SOD) and Catalase (CAT) activities were not affected by the HP plus valerian treatment in the liver and kidney of rats. HP and valerian when administered independently did not affect the activity of hepatic and renal δ-aminolevulinate dehydratase (δ-ALA-D), however, these drugs administered concomitantly provoked an inhibition of hepatic δ-ALA-D activity. The δ-ALA-D reactivation index was higher in rats treated with HP plus valerian than other treated groups. These results strengthen the view that δ-ALA-D can be considered a marker for oxidative stress. Serum aspartate aminotransferase (AST) activity was not altered by any treatment. However, serum alanine aminotransferase (ALT) activity was higher in the HP group and HP plus valerian group. Our findings suggest adverse interactions between haloperidol and valerian.

1. Introduction

Haloperidol (HP) is a drug with useful properties in the management of psychosis (Creese et al., 1976). The long-term use of HP, however, is associated with extrapyramidal side effects, including tardive dyskinesia (See and Ellison, 1990). This syndrome is characterized by involuntary orofacial movements and is often irreversible even after drug withdrawal (Gerlach and Casey, 1988; Glazer et al., 1990; Andreasen and Jorgensen, 2000). The persistence of tardive dyskinesia after the discontinuation of HP treatment suggests that this condition may be related to a neuronal lesion induced by HP or a reactive metabolite(s) derived from its metabolism (Wright et al., 1998).

In line with this, HP can be metabolized to the toxic intermediate 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)-4-oxobutyl]pyridinium) (HPP^+) in the liver (Subramanyam et al., 1991; Bloonquist et al., 1994; Rollema et al., 1994; Fang et al., 1995). This reactive metabolite is thought to share some toxic similarities with the neurotoxic agent 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) and can be involved in the genesis of some Parkinson-like effects associated with the chronic use of HP (Ablordeppey and Borne, 1993).

Valerian root (*Valeriana officinalis* L.) is the most commonly used herbal product to induce sleep in both the United States and Europe (Richman and Witkowski, 1998; 1999; Houghton, 1999; Ang-Lee et al., 2001; Malva et al., 2004). Virtually all sleep-aid herbal dietary supplements contain valerian (Ang-Lee, 2003). The pharmacological activities of valerian are attributed to the

different constituents, including valepotriates (valtrate/ isovaltrate and dihydrovaltrate) (Von der Hude et al., 1985) and valerenic acid. Valerian or its constituents could induce these effects by interacting with central gamma-aminobutyric acid (GABA) receptors (Mennini et al., 1993; Cavadas et al., 1995). In vitro studies testing the binding of valerian extract to GABA receptors showed that the agonist muscimol was displaced, suggesting valerian binding to these receptors (Cavadas et al., 1995). Valerenic acid has been shown to inhibit enzyme-induced breakdown of GABA in the brain resulting in sedation (Houghton, 1999). Recently data showed the inductive effect of valerian on cytochrome P450 (CYP) 3A4 and 2D6 enzymes in vitro (Hellum et al., 2006). This should be seen with caution since alterations of CYP activity could affect the disposition of conventional pharmaceuticals (Gonzalez, 2005). This is a major problem in human therapy especially with CYP3A4 involved in metabolism of over 60% of all therapeutically used drugs (Wrighton et al., 2000).

Reports of drug interaction with many botanicals, including valerian have been noted in literature (Assemi, 2001; Brazier and Levine, 2003). Natural products can interact with drugs by affecting the biological processes that regulate their metabolism and elimination (Bailey and Dresser, 2004). The widespread use of valerian supplements suggests that use with conventional medications is inevitable, and the potential for drug interactions is undefined (Fugh-Berman and Ernst, 2001).

HP was used in this study for the reason that it is extensively use in clinical practice to treat psychotic disorders and severe behavioral problems, and because the side effects of HP on the CNS could be attributed to toxic metabolites and/or oxidative stress (Wright et al., 1998; Burger et al., 2005). Also, other side effects of HP like anxiety, restlessness, agitation and insomnia are of interest for this work since valerian major effect is sedation (Malva et al., 2004). Long-term safety studies with valerian are lack in the literature (Hadley and Petry, 2003). In view of the fact that valerian has been reported to affect the metabolism of other drugs, considering that haloperidol is extensively metabolized in the liver and used in long-term treatments (Forsman et al., 1977), the present study was undertaken to determine whether the treatment of haloperidol, valerian or both in association can affect the liver or kidney of rats in a long-term study.

2. Materials and Methods

2.1. Chemicals

Haloperidol Decanoate was donated by Cristália (São Paulo, Brazil). A standard tincture of *Valeriana officinalis* (10 g of valerian roots per 100 mL of ethanol) was obtained from Bio extracts (São Paulo, Brazil). *O*-phthalaldehyde, thiobarbituric acid and DL-dithiothreitol (DTT) were obtained from Sigma (St. Louis, MO, USA). $HgCl_2$, NaCl, K_2HPO_4, KH_2PO_4, trichloroacetic acid, *para*-dimethylaminobenzaldehyde, and glacial acetic acid were purchased from

Reagen (Rio de Janeiro, RJ, Brazil). All other chemicals were purchased from Merck (Darmstadt, Germany).

2.2. Animals

Male Wistar rats weighing 295 (± 35.36) g with approximately 3 months of age, from our own breeding colony were kept in cages with continuous access to food and *V. officinalis* or its vehicle (ethanol 1%), in a room with controlled temperature (22±3°C) and in 12h light/ dark cycle with lights on at 7:00 am. The animals were maintained and used in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of Federal University of Santa Maria, Brazil.

2.3. Treatments

Treatment consisted of 1% ethanol (vehicle of *V. officinalis*) or 1% (final concentration of 100 mg/mL) of a standard tincture of *V. officinalis* (10 g of valerian roots per 100 mL of ethanol) in the drinking water. The dosage was calculated every week by the amount of water drunk assuming equal drinking among the four animals in each cage. Thus, each animal received *V. officinalis* extract in a dosage about 200-250 mg/Kg/day. The doses of valerian were based on the daily recommended dose for an adult human which is 3060 mg (as inscribed on the commercially bottle of valerian by Nature's way product). These solutions were placed daily before the beginning of the dark cycle. It was not observed a reduction in liquid intake. Haloperidol Decanoate or its vehicle

(soy oil) were administered intramuscularly (i.m.) every 28 days (38 mg/kg) that is equivalent to 1mg/kg/day of unconjugated HP (Fachinetto et al., 2005). *V. officinalis* treatment started 15 days before the administration of HP. The treatment with haloperidol was carried out during 12 weeks concomitantly with *V. officinalis*. This treatment was performed according to a previous study of Fachinetto et al. (2007).

Animals were divided into four groups of 12 animals each:

1 -Control: received soy oil (i.m.) and 1% of ethanol in the drink water;

2 -Valerian: received soy oil (i.m.) and 1% of *V. officinalis* in the drink water;

3 -HP: received Haloperidol Decanoate (i.m.) and 1% of ethanol in the drink water;

4 -Combined treatment: received Haloperidol Decanoate (i.m.) and 1% of *V. officinalis* in the drink water.

2.4. Tissue preparation

Animals were euthanized by decapitation. Liver and kidney were quickly removed, placed on ice and homogenized at 7 and 5 volumes of 0.9% NaCl, respectively. The homogenates were centrifuged at 4,000 x g for 10 min to yield a low-speed supernatant fraction (S1) that was used for the biochemical and enzymatic assays, (except for the GSH/GSSG assay). In order to perform SOD and CAT assay, S1 was diluted as described in the respective sections (2.7.2 and 2.7.3).

2.5. Lipid peroxidation assay

Thiobarbituric acid reactive species (TBARS) were determined as described by Ohkawa et al. (1979). In brief, samples were incubated at 100 ºC for 1 h in a medium containing 8.1 % sodium dodecyl sulfate, 1.4 M acetic acid, pH 3.4 and 0.6% thiobarbituric acid. The pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde (MDA), a secondary product of lipid peroxidation, was measured spectrophotometrically at 532 nm. Standard curve of MDA was used in order to calculate MDA concentrations. Results were expresses as nmol of MDA/ mg of protein.

2.6. Estimation of reactive species production

Formation of reactive species was estimated according to a previous report (Ali et al., 1992). Liver and kidney tissue samples were homogenized in 2.2 mL of saline solution (0.9% NaCl). Aliquots of 2.5 mL were incubated in the presence of 5 μM 2',7'-dichlorofluorescein diacetate at 37 ºC for 60 min. Fluorescent signals were recorded at the end of the incubation at an excitation wavelength of 488 nm and an emission wavelength of 525 nm. Results were expressed as fluorescence units.

2.7. Enzyme assays

2.7.1. δ-ALA-D activity

δ-ALA-D activity was assayed according to the method of Sassa (1982) by measuring the rate of product (porphobilinogen-PBG) formation. The reaction product was determined using modified Ehrlich's reagent at 555 nm,

with a molar absorption coefficient of 6.1×10^4 M^{-1} for the Ehrlich-PBG salt. The incubation medium contained δ-ALA 2.4 mM and potassium phosphate buffer (pH 6.8) 0.084 M. The reaction was initiated by the addition of enzymatic material and the incubations were carried out for 90 and 150 minutes, for liver and kidney respectively, at 39°C. Afterwards, the reaction was stopped by the addition of trichloroacetic acid 10% containing $HgCl_2$ 0.01 M. The activity of δ-ALA-D was expressed as nmol of PBG/ mg of protein/ h. Simultaneously, a set of tubes was assayed using the same protocol, except that 2 mM DTT was added in order to obtain the reactivation index. This index indicates the extent of the reactivation of δ-ALA-D activity. The reactivation index of δ-ALA-D activity was calculated as follows:

$$\frac{(\delta\text{-ALA-D activity with DTT} - \delta\text{-ALA-D activity without DTT})}{\delta\text{-ALA-D activity with DTT}} \times 100\%$$

2.7.2. SOD activity

S1 of kidney and liver was adequately diluted with 40 and 60 volumes of 0.9% NaCl, respectively, in order to perform the SOD assay (Misra and Fridovich, 1972). Briefly, epinephrine undergoes auto-oxidation at pH 10.2 to produce adrenochrome, a colored product that was detected at 480 nm. The addition of samples (10, 20, 30 µL) containing SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored during 180 seconds. The amount of enzyme required to produce 50% inhibition was defined as one unit of enzyme activity.

2.7.3. CAT activity

CAT activity was measured by the method of Aebi (1974). An aliquot of liver and kidney supernatants (10 µL) diluted with 60 and 40 volumes of 0.9% NaCl, respectively, was added to a quartz cuvette and the reaction was started by the addition of freshly prepared H_2O_2 (30 mM) in phosphate buffer (50 mM, pH 7). The rate of H_2O_2 decomposition was measured spectrophotometrically at 240 nm during 120 seconds. The activity of CAT was expressed as µmol H_2O_2/mg protein/min.

2.7.4. Serum transaminases

Serum enzymes, AST and ALT were used as biochemical markers of hepatic damage, using a commercial Kit (LABTEST, Diagnostica S.A., Minas Gerais, Brazil) (Reitman and Frankel, 1957).

2.8. Fluorometric assay of reduced (GSH) and oxidized (GSSG) glutathione

For measurement of GSH and GSSG levels, we used a method previously described by Hissin and Hilf (1976). Briefly, 250 mg of the tissue was homogenized in 3.75 mL phosphate-EDTA buffer (pH 8) plus 1 mL HPO_3 (25%). Homogenates were centrifuged at 4°C at 100,000 g for 30 min and the supernatant were separated in two different aliquots for measurement of GSH and GSSG.

For GSH measurement, 500 µL of the supernatant was added to 4.5 mL of phosphate buffer. The final assay mixture (2.0 mL) contained 100 µL of the diluted tissue supernatant, 1.8 µL of phosphate buffer, and 100 µL of *o*-

phthalaldehyde (1 μg/μL). The mixtures were incubated at room temperature for 15 min and their fluorescent signals were recorded in the luminescence spectrometer at 420 nm of emission and 350 nm of excitation wavelengths.

For measurement of GSSG levels, a 500 μL portion of the original supernatant was incubated at room temperature with 200 μL of *N*-ethylmaleimide (NEM) (0.04 M) for 30 min to react with free GSH to prevent its oxidation to GSSG. To this mixture, 4.3 μL of NaOH (0.1 N) was added. A 100-μL portion of this mixture was taken for measurement of GSSG, using the procedure outlined above for GSH assay, except that NaOH was employed as diluent rather than phosphate-EDTA buffer. Results were expressed as GSH/GSSG ratio.

2.9. Protein measurement

Protein was assayed by the method of Lowry et al. (1951), with bovine serum albumin as standard.

2.10. Statistical analysis

Data were analyzed statistically by one-way ANOVA, followed by Duncan's multiple range test when appropriate. The results were considered statistically significant for $p<0.05$.

3. Results

Lipid Peroxidation

TBARS levels in liver homogenates were not affected by HP or valerian when compared to the control (Fig. 1A). However, in the combined treatment group a significant increase in TBARS production (about 15%) was detected when compared to the other groups ($p<0.05$, Fig. 1A). Renal TBARS levels were not modified by any of the treatments (Fig. 1B). <Insert figure 1 here>

Estimation of reactive species production

Reactive species production was measured by DCFH oxidation. Combined treatment significantly enhanced the oxidation of DCFH (about 70%) in liver homogenates when compared to the control group (Fig. 2A, $p<0.05$). HP and valerian alone did not alter hepatic reactive species production (Fig. 2A); however, it is worth mentioning that they may have had an additive effect on DCFH oxidation in liver. In kidney homogenates, DCFH oxidation was not modified by any treatment (Fig. 2B). <Insert figure 2 here>

Enzyme activity

Hepatic and renal SOD activities were not altered by HP, valerian or combined treatment (data not shown). CAT activity was not modified by any of treatments in both organs (data not shown). Isolated HP and valerian treatment did not affect hepatic or renal δ-ALA-D activity (Fig. 3A and 4A). <Insert figure 3 here> However, HP in combination with valerian showed an inhibitory effect on δ-ALA-D activity in the liver (Fig 3A, $p<0.05$) but not in the kidney (Fig 4A). In vitro, DTT, a classic agent that restores oxidized thiol groups, was able to restore liver δ-ALA-D activity (Fig 3B). In fact, the combined treatment of HP

and valerian had the highest δ-ALA-D reactivation index (Fig 3B, $p<0.05$). In the kidney, there was no difference in the δ-ALA-D reactivation index among groups (Fig 4B). <Insert figure 4 here>

Serum AST activities were not modified by any treatment (Figure 5A). Rats treated with HP presented an increase in serum ALT activities when compared to control rats (Figure 5B, $p<0.05$). The valerian treatment did not significantly alter serum ALT activities, although its association with HP statistically increased this parameter (Figure 5B, $p<0.05$). The magnitude of the increase observed in serum ALT activities deserves attention however it was not high enough to be of clinical concern. <Insert figure 5 here>

GSH and GSSG

The HP treatment provoked a decrease in the GSH/GSSG ratio in liver homogenates and the valerian concomitant treatment did not avoid the HP effects (Fig. 6A, $p<0.05$). In fact valerian it self had no effect on the, GSH/GSSG ratio. In the kidney, none of the treatments modified the GSH/GSSG ratio (Fig. 6B). <Insert figure 6 here>

4. Discussion

The long-term use of HP is associated to side effects such as Parkinsonism and tardive dyskinesia, and these syndromes have been attributed to a toxic metabolite of HP, the pyridinium metabolite HPP^+ (Wright et

al., 1998). Nevertheless, there are few reports demonstrating the toxic effects of HP on the liver or kidney. Since haloperidol is widely used in clinical practice, the knowledge of its toxicity is essential in the choice of this antipsychotic agent. The results presented here showed that HP alone only altered one of the oxidative stress parameters in the liver (GSH/GSSG) and none in the kidney. On the other hand, when HP was associated to valerian, a significant increase in TBARS levels and DCFH reactive species production was observed in the hepatic tissue. Our results indicate an increase in oxidative damage evoked by HP plus valerian.

Despite the oxidative damage, SOD and CAT activities were not affected by combined treatment in the liver. Similarly, HP failed to alter plasma SOD and CAT activities in patients taking this drug (Yao et al., 1998). Considering that HP is extensively metabolized in the liver with only approximately 1% of the administered dose excreted in the urine (Forsman et al., 1977), we were not surprised that this treatment did not cause any deleterious effects on kidney from rats of this study.

Valeriana officinalis is widely utilized for its sedative properties, although there is a lack in the literature of long-term safety studies with valerian (Hadley and Petry, 2003). In our study, valerian alone did not show any effect on TBARS levels, DFCH production or SOD and CAT activities. Al-Majed and colleagues (2006) reported an increase in the concentrations of MDA and a decrease of non-protein sulfhydryl levels in hepatic cells of mice

after sub-acute treatment with valerian. However, in this study we only observed side effects when valerian was associated to HP.

δ-ALA-D activity was tested here since it is a sulfhydryl-containing enzyme that is susceptible to oxidizing agents and is inhibited after exposure to pro-oxidant situations (Folmer et al., 2002, 2003; Soares et al., 2003; Gonçalves et al., 2005; Santos et al., 2005). The inhibition of δ-ALA-D may impair heme biosynthesis and can result in the accumulation of aminolevulinic acid, which has been demonstrated to be a pro-oxidant molecule under significant physiological conditions (Bechara et al., 1993; Bechara, 1996; Emanuelli et al., 2001). HP and valerian when administered separately did not affect the activity of δ-ALA-D, however, these drugs administered in association provoked an inhibition of hepatic δ-ALA-D activity. This is supported by the δ-ALA-D reactivation index which presented the highest level in the combined treatment when compared to the other groups. HP and valerian could be interacting in a manner that oxidizes δ-ALA-D sulfhydryl groups, probably by producing some oxidative by-products. These data are in agreement with the fact that DTT, a classic reducing agent that restores oxidized thiol containing enzymes (Perottoni et al., 2005), could restore the inhibition caused by the combined treatment. In fact, it has been reported that valtrate, a component of valerian, may interact with free sulfhydryl groups (Keochanthala-Bounthanh et al., 1990). Perhaps HP can enhance this capacity of valerian to interact with

sulfhydryl groups or affect the susceptibility of the liver. These results strengths the view that δ-ALA-D can be considered a marker for oxidative stress.

The release of intracellular enzymes (AST and ALT), which is presumed to be a result of injury to cellular membranes, was utilized here as an index of hepatotoxicity. Serum AST activity was not altered by any treatment. However, serum ALT activity was higher in the HP and combined treatment. Gaertner and collaborators (2001) reported elevation in transaminases in patients treated with HP, which support these results. Anyway the increase in ALT activity observed here was small to be of clinical concern.

In our study, HP caused a decrease in the GSH/GSSG index in the liver, which was also observed in the HP and valerian combined treatment. This decrease, which lowers cellular defenses, can contribute to the increase in TBARS and DCFH oxidation, and lead to the elevations observed in serum ALT activity.

The results present here point to an interaction between HP and valerian in the increase of oxidative stress in the liver. Gold and colleagues (2001) identified a series of potential interactions between herbal natural health products, including valerian, and conventional drug therapies. Herb–drug interactions can appear when herbs and chemical drugs are co-administered and the herbal preparation (one or more components) modulates the metabolism of the chemical drug by induction or inhibition of CYP enzymes. Hellum and collaborators (2006) showed the capacity of valerian extracts to

induce CYP enzymes in cultured primary human hepatocytes, including CYP3A4, the most important P450 enzyme responsible for HP metabolic pathways (Igarashi et al., 1995; Usuki et al., 1996; Fang et al., 2001; Kalgutkar et al., 2003; Avent et al., 2006). Based on these reports, we suppose that the mechanism by which valerian-HP association induced oxidative damage may be related to CYP activity. The inducible effects of valerian on CYP enzymes could increase the production of toxic HP metabolites, such as HPP^{+}, responsible for the deleterious effects seen in the valerian plus HP treatment in the liver. The adverse effects of valerian plus HP treatment observed in the liver and the absence of these effects in kidney also could be explaining by this mechanism. CYP enzymes are found at highest levels in the liver, and expressed at lower levels in kidney (Gonzalez, 2005). Thus, liver are the major site of herb-drug interaction in which the production of toxic HP metabolites could be elevated.

The route of administration of Haloperidol Decanoate (i.m.) as well as the dose of HP used in this study was equivalent to that indicated for humans which increase the relevance of this work since our model could be translated into the human situation. The doses of valerian and HP used here was within the normal range for humans, but a possible negative interaction between these drugs will occur in a real human situation only after ingestion of supratherapeutic doses of valerian and HP. Anyway a possible toxic additive effect of these two compounds must be kept in mind. In this way, the administration of valerian in

people taking HP should be better studied to verify possible negative interactions.

The use of herbs as alternative and/or complementary therapy in the Western world is on the rise and gaining increasing popularity (Hellum et al., 2006). Unfortunately, their use has often been accompanied by an unfounded belief that “natural” is equal to “safe”. The widespread use of valerian supplements suggests that its use with conventional medications is inevitable. Moreover, the potential for drug interactions with natural products is undefined (Fugh-Berman and Ernst, 2001). The findings from our study suggest an adverse effect in the interactions between haloperidol and valerian causing hepatic damage related to oxidative stress. However, further work is needed to confirm the exact mechanism by which these compounds cause oxidative stress. Moreover, caution is warranted if attempting to make such an extrapolation to other herbals and drug therapies.

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References

Ablordeppey, S.Y., Borne, R.F., 1993. Detection of a neurotoxic quaternary pyridinium metabolite in the liver of haloperidolnext term-treated rats. Pharmacol. Biochem. Behav. 46, 739-744.

Aebi H., 1974. Catalase, In: Bergmeyer, H.V. (Ed.), Methods of Enzymatic Analysis. VEB Chemie Weinheim, pp. 673-677.

Ali, S.F., LeBel, C.P., Bondy, S.C., 1992. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. Neurotoxicology 13, 637-648.

Al-Majed, A.A., Al-Yahya, A.A., Al-Bekairi, A.M., Al-Shabanah, O.A., Qureshi, S., 2006. Studies on the cytological and biochemical effects of valerian in somatic and germ cells of Swiss albino mice. Food Chem. Toxicol. 44, 1830-1837.

Andreasen, O.A., Jorgensen, H.A., 2000. Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats. Implications for tardive dyskinesia? Prog. Neurobiol. 61, 525-541.

Ang-Lee, M.K., Moss, J., Yuan, C.S., 2001. Herbal medicines and perioperative care. JAMA 286, 208-216.

Ang-Lee, M.K., 2003. Most commonly used herbal medicines in the U.S.A., In: Yuan, C.S. and Bieber, E.J. (Eds), Textbook of complementary and alternative medicine. Parthenon, New York, pp. 7–32.

Assemi, M., 2001. Herbs affecting the central nervous system: gingko, kava, St. John's wort, and valerian. Clin. Obstet. Gynecol. 48, 824-835.

Avent, K.M., DeVoss, J.J., Gillam, E.M., 2006. Cytochrome P450-mediated metabolism of haloperidol and reduced haloperidol to pyridinium metabolites. Chem. Res. Toxicol. 19, 914-920.

Bailey, D.G., Dresser, G.K., 2004. Natural products and adverse drug interactions. CMAJ 170, 1531-1532.

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

Bechara, E.J., 1996. Oxidative stress in acute intermittent porphyria and lead poisoning may be triggered by 5-aminolevulinic acid. Braz. J. Med. Biol. Res. 29, 841-51.

Bloomquist, J., King, E., Wright, A., Mytilineou, C., Kimura, K., Castagnoli, K., Castagnoli Jr, N., 1994. 1-Methyl-4-phenylpyridinium-like neurotoxicity of a pyridinium metabolite derived from haloperidol: cell culture and neurotransmitter uptake studies. J. Pharmacol. Exp. Ther. 270, 822-830.

Brazier, N.C., Levine, M.A., 2003. Drug-herb interaction among commonly used conventional medicines: a compendium for health care professionals. Am. J. Ther. 10, 163-169.

Burger, M.E., Fachineto, R., Alves, A., Callegari, L., Rocha, J.B., 2005. Acute reserpine and subchronic haloperidol treatments change synaptosomal brain glutamate uptake and elicit orofacial dyskinesia in rats. Brain Res. 1031, 202-210.

Cavadas, C., Araujo I., Cotrim, M.D., Amaral, T., Cunha, A.P., Macedo, T., Ribeiro, C.F., 1995. In vitro study on the interaction of Valeriana officinalis L. extracts and their amino acids on GABAA receptor in rat brain. Arzneimittelforschung 45, 753-755.

Creese, I., Burt, D.R., Snyder, S.H., 1976. Dopamine receptor binding predicts clinical and pharmacological potencies of anti-schizophrenic drugs. Science 192, 481-483.

Emanuelli, T., Pagel, F.W., Alves, L.B., Regner, A., Souza, D.O., 2001. Inhibition of adenylate cyclase activity by 5- aminolevulinic acid in rat and human brain. Neurochem. Int. 38, 213-218.

Fachinetto, R., Burger, M.E., Wagner, C., Wondracek, D.C., Brito, V.B., Nogueira, C.W., Ferreira, J., Rocha, J.B., 2005. High fat diet increases the incidence of orofacial dyskinesia and oxidative stress in specific brain regions of rats. Pharmacol. Biochem. Behav. 81, 585-592.

Fachinetto, R., Villarinho, J., Wagner, C., Pereira, R.P., Ávila, D. S., Burger, M.E., Calixto, J.B., Rocha, J.B.T., Ferreira, J., 2007. *Valeriana officinalis* does not alter the orofacial dyskinesia induced by haloperidol in rats: role of

dopamine transporter. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 1478-1486.

Fang, J., Zuo, D., Yu, P.H., 1995. Comparison of cytotoxicity of a quaternary pyridinium metabolite of haloperidol (HP+) with neurotoxin N-methyl-4-phenylpyridinium (MPP+) towards cultured dopaminergic neuroblastoma cells. Psychopharmacology (Berl.) 121, 373-378.

Fang, J., Mckay, G., Song, J., Remillrd, A., LI, X., Midha, K., 2001. In vitro characterization of the metabolism of haloperidol using recombinant cytochrome P450 enzymes and human liver microsomes. Drug Metab. Dispos. 12, 1638–1643.

Folmer, V., Soares, J.C., Rocha, J.B.T., 2002. Oxidative stress in mice is dependent on the free glucose content of the diet. Int. J. Biochem. Cell Biol. 34, 1279-1285.

Folmer, V., Soares, J.C., Gabriel, D., Rocha, J.B.T., 2003. A high fat diet inhibits delta-aminolevulinate dehydratase and increases lipid peroxidation in mice (Mus musculus). J. Nutr. 133, 2165-2170.

Forsman, A., Folsch, G., Larsson, M., Ohman, R., 1977. The metabolism of haloperidol in man. Curr. Ther. Res. Clin. Exp. 21, 606-617.

Fugh-Berman, A., Ernst, E., 2001. Herb-drug interactions: review and assessment of report reliability. Br. J. Clin. Pharmacol. 52, 587-595.

Gaertner, I., Altendorf, K., Batra, A., Gaertner, H.J., 2001. Relevance of liver enzyme elevations with four different neuroleptics: a retrospective review of 7,263 treatment courses. J. Clin. Psychopharmacol. 21, 215-222.

Gerlach, J., Casey, D., 1988. Tardive dyskinesia. Acta Psychiatr. Scand. 77, 369-378.

Glazer, W.M., Morgenstern, H., Shooler, N., Berkman, C.S., Moore, D.C., 1990. Predictors of improvement in tardive dyskinesia following discontinuation of neuroleptic medication. Br. J. Psychiatry 157, 585-592.

Gold, J.L., Laxer, D.A., Dergal, J.M., Lanctot, K.L., Rochon, P.A., 2001. Herbal-drug therapy interaction: a focus on dementia. Curr. Opin. Clin. Nutr. Metab. Care 4, 29-34.

Gonçalves, T.L., Erthal, F., Corte, C.L.D., Muller, L.G., Piovezan, C.M., Nogueira, C.W., Rocha, J.B.T., 2005. Involvement of oxidative stress in the pre-malignant and malignant states of cervical cancer in women. Clin. Biochem. 38, 1071-1075.

Gonzalez, F.J., 2005. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. Mutat. Res. 569, 101-110.

Hadley, S., Petry, J.J., 2003. Valerian. Am. Fam. Physician 67, 1755-1758.

Hellum, B.H., Hu, Z., Nilsen, O.G., 2006. The Induction of CYP1A2, CYP2D6 and CYP3A4 by six trade herbal products in cultured primary human hepatocytes. Basic Clin. Pharmacol. Toxicol. 100, 23–30.

Hissin, P.J., Hilf, R., 1976. A fluorimetric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74, 214–226.

Houghton, P.J., 1999. The scientific basis for the reputed activity of valerian. J. Pharm. Pharmacol. 51, 505-512.

Igarashi, K., Kasuya, F., Fukui, M., Usuki, E., Castagnoli Jr., N., 1995. Studies on the metabolism of haloperidol (HP): the role of CYP3A in the production of the neurotoxic pyridinium metabolite HPP^+ found in rat brain following IP administration of HP. Life Sci. 57, 2439-2446.

Kalgutkar, A.S., Taylor, T.J., Venkatakrishnan, K., Isin, E.M., 2003. Assesment of the contributions of CYP3A4 and CYP3A5 in the metabolism of the antpsychotic agent haloperidol to its potencially neurotoxin pyridinium metabolite and effect of antidepressants on the bioactivation pathway. Drug Metab. Dispos. 31, 243-249.

Keochanthala-Bounthanh, C., Haag-Berrurier, M., Beck, J.P., Anton, R., 1990. Effects of thiol compounds versus the cytotoxicity of valepotriates on cultured hepatoma cells. Planta Med. 56, 190-192.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Malva, J.O., Santos, S., Macedo, T., 2004. Neuroprotective properties of Valeriana officinalis extracts. Neurotox. Res. 6, 131–140.

Mennini, T., Bernasconi, P., Bombardelli, E., Morazzoni, P., 1993. In vitro study on the interaction of extracts and pure compounds from *Valeriana officinalis*

roots with GABA, benzodiazepine and barbiturate receptors in rat brain. Fitoterapia 64, 291–300

Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170–3175.

Ohkawa, H., Ohishi, H., Yagi, K., 1979. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351-358.

Perottoni, J., Meotti, F.C., Folmer, V., Pivetta, L., Nogueira, C.W., Zeni, G., Rocha, J.B.T., 2005. Ebselen and diphenyl diselenide do not change the inhibitory effect of lead acetate on delta aminolevulinate dehydratase. Environ. Toxicol. Pharmacol. 19, 239-248.

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.

Richman, A., Witkowski, J.P., 1998. 4th Annual Herbal Products Sales Survey, Whole Foods 21, 19–26.

Richman, A., Witkowski, J.P., 1999. 5th Annual Herbal Products Sales Survey, Whole Foods 22, 49-56.

Rollema, H., Skolnik, M., D'Engelbronner, J., Igarashi, K., Usuki, E., Castagnoli Jr, N., 1994. MPP(+)-like neurotoxicity of a pyridinium metabolite derived from haloperidol: in vivo microdialysis and in vitro mitochondrial studies. J. Pharmacol. Exp. Ther. 268, 380-387.

Santos, F.W., Zeni, G., Rocha, J.B.T., Nascimento, P.C., Marques, M.S., Nogueira, C.W., 2005. Efficacy of 2,3-dimercapto-1-propanesulfonic acid (DMPS) and diphenyl diselenide on cadmium induced testicular damage in mice. Food Chem. Toxicol. 43, 1723-1730.

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

See, R.E., Ellison, G., 1990. Intermittent and continuous haloperidol regimens produce different types of oral dyskinesias in rats. Psychopharmacology (Berl.) 100, 404–412.

Soares, J.C., Folmer, V., Rocha, J.B.T., 2003. Influence of dietary selenium supplementation and exercise on thiol-containing enzymes in mice. Nutrition 19, 627-632.

Subramanyam, B., Woolf, T., Castagnoli Jr, N., 1991. Studies on the in vitro conversion of haloperidol to a potentially neurotoxic pyridinium metabolite. Chem. Res. Toxicol. 4, 123-128.

Usuki, E., Pearce, R., Parkinson, A., Castagnol Jr, N., 1996. Studies on the conversion of haloperidol and its tetrahydropyridine dehydration product to potentially neurotoxic pyridinium metabolites by human liver microsomes. Chem. Res. Toxicol. 9, 800-806.

Von der Hude, W., Scheutwinkel-Reich, M., Braun, R., Dittmar, W., 1985. In vitro mutagenicity of valepotriates. Arch. Toxicol. 56, 267–271.

Yao, J.K., Reddy, R., McElhinny, L.G., van Kammen, D.P., 1998. Effects of haloperidol on antioxidant defense system enzymes in schizophrenia. J. Psychiatr. Res. 32, 385-391.

Wright, A.M., Bempong, J., Kirby, M.L., Barlow, R.L., Bloomquist, J.R., 1998. Effects of haloperidol metabolites on neurotransmitter uptake and release: possible role in neurotoxicity and tardive dyskinesia. Brain Res. 788, 215-222.

Wrighton, S.A., Schuetz, E.G., Thummel, K.E., Shen, D.D., Korzekwa, K.R., Watkins, P.B., 2000. The human CYP3A subfamily: practical considerations. Drug Metab. Rev. 32, 339-361.

Legends

Figure 1. Effects of valerian and/ or HP treatments on TBARS production in liver (A) and kidney (B) homogenates. Data are expressed as mean ± S.E.M. for twelve rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 2. Effects of valerian and/ or HP treatments on DCFH oxidation in liver (A) and kidney (B) homogenates. Data are expressed as mean ± S.E.M. for twelve rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 3. Effects of valerian and/ or HP treatments on δ-ALA-D activity in liver homogenates (A) and on the enzyme reactivation index (B). Data are expressed as mean ± S.E.M. for twelve rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 4. Effects of valerian and/ or HP treatments on δ-ALA-D activity in kidney homogenates (A) and on the enzyme reactivation index (B). Data are expressed as mean ± S.E.M. for twelve rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 5. Effects of valerian and/ or HP treatments on serum AST (A) and ALT (B) activities. Data are expressed as mean ± S.E.M. for twelve rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 6. Effects of valerian and/ or HP treatments on GSH/GSSG ratio in liver (A) and kidney (B) homogenates. Data are expressed as mean ± S.E.M. for twelve rats per group. Experiments were performed in duplicates. (*) represents $p<0.05$ as compared to control group by Duncan's multiple range test.

Figure 1.

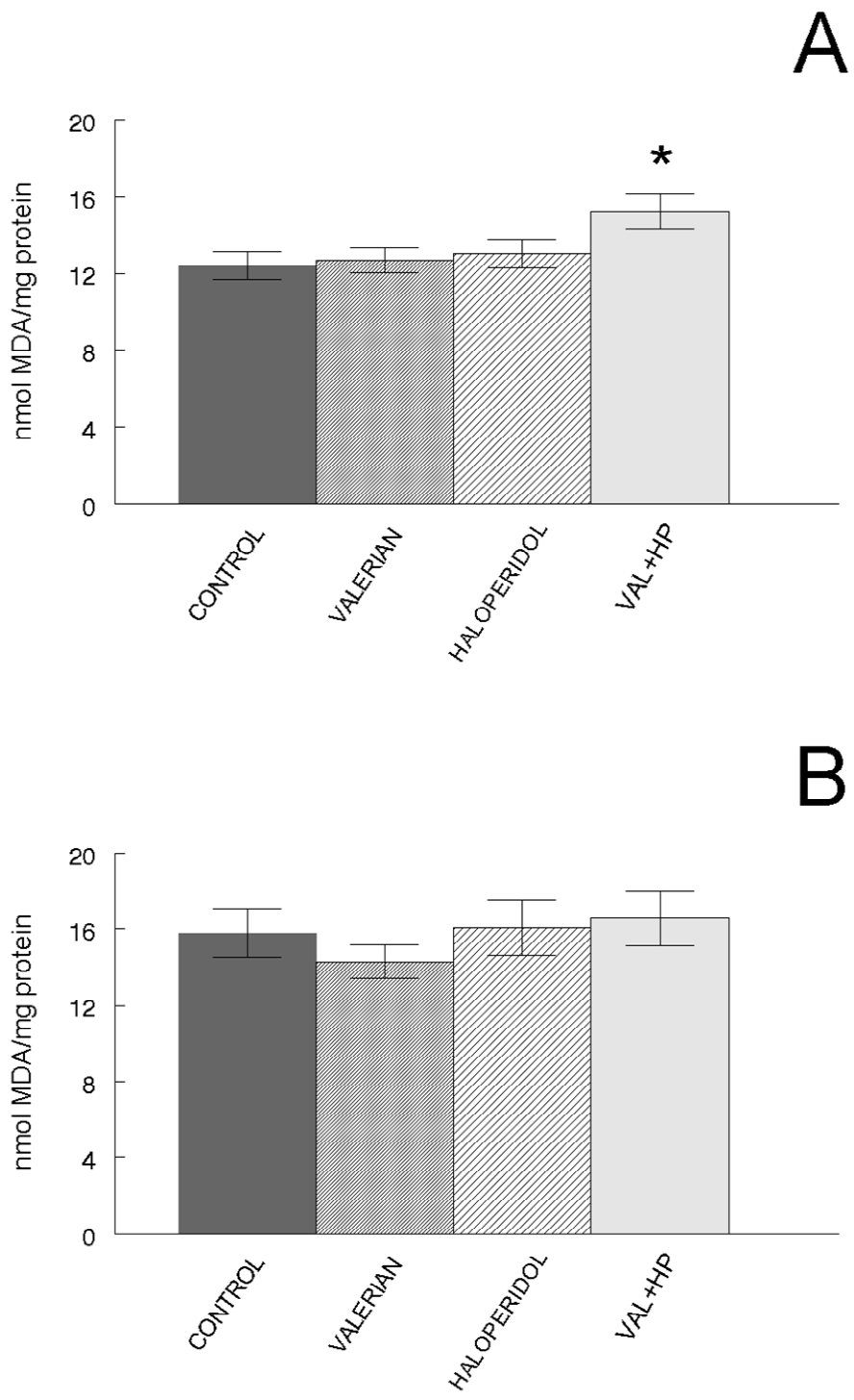

A
nmol MDA/mg protein
20
16
12
8
4
0
*
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP
B
nmol MDA/mg protein
20
16
12
8
4
0
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP

Figure 2.

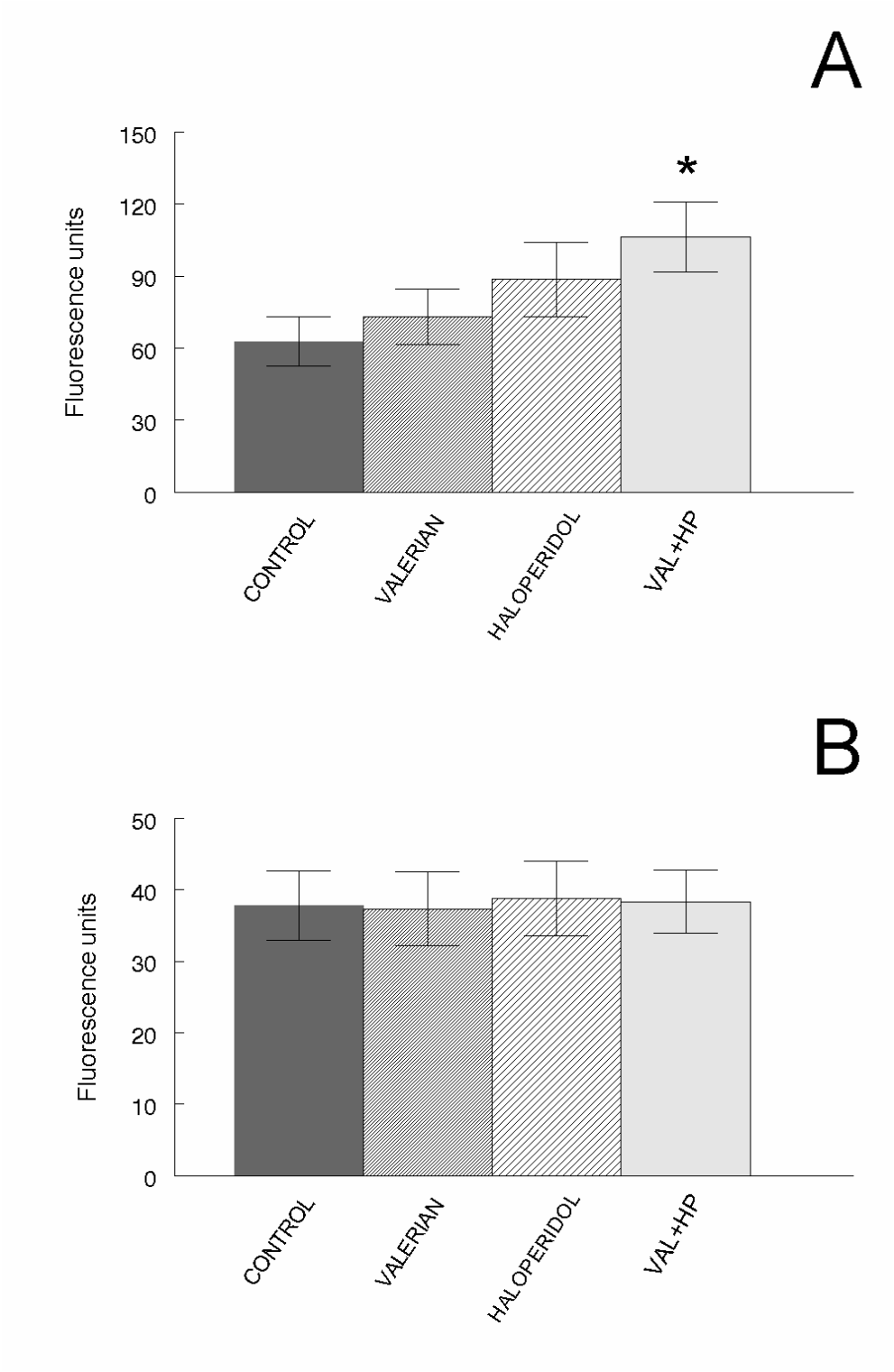

A
150
120
90
60
30
0
Fluorescence units
*
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP
B
50
40
30
20
10
0
Fluorescence units
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP

Figure 3.

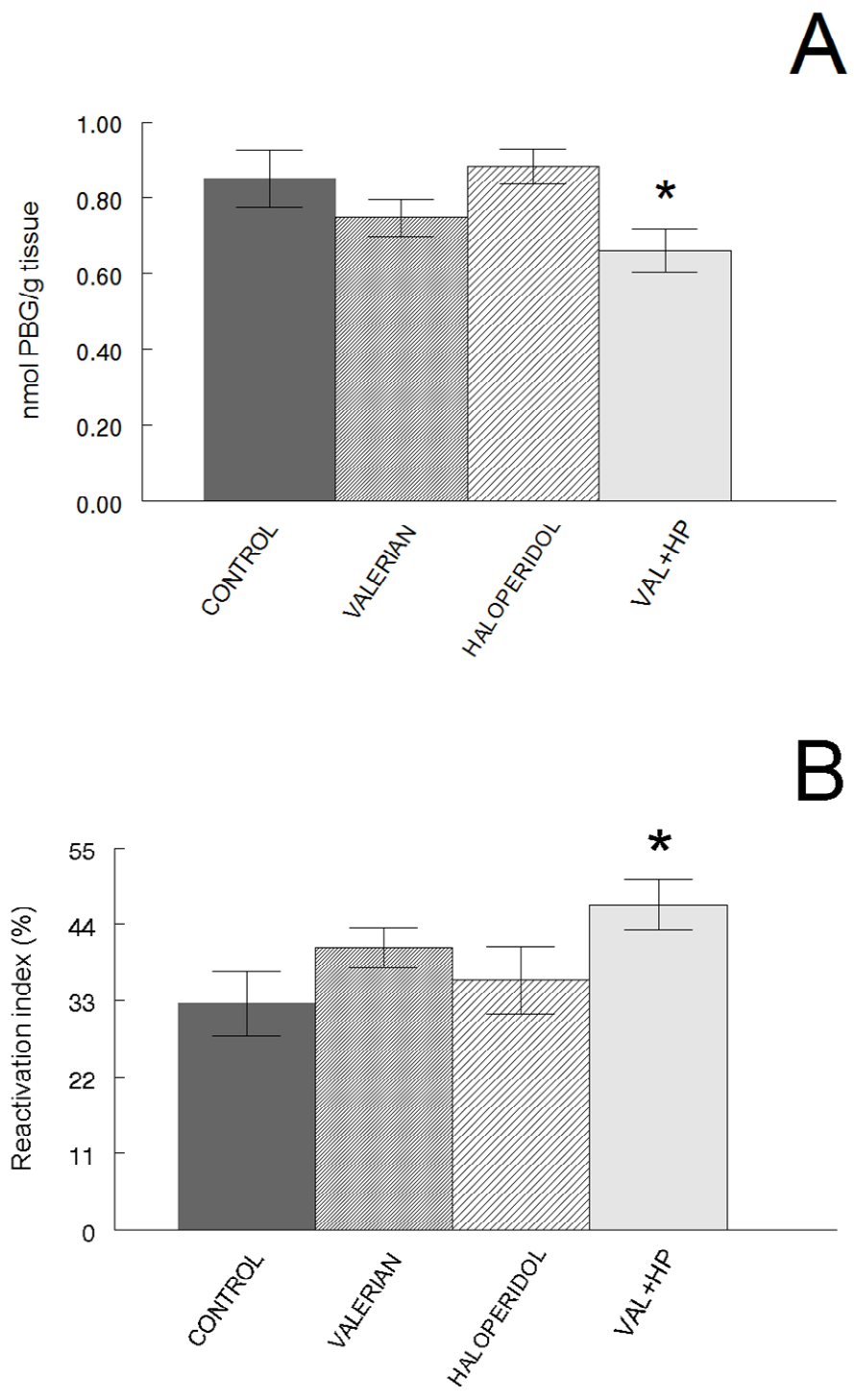

A
1.00
0.80
0.60
0.40
0.20
0.00
nmol PBG/g tissue
*
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP
B
55
44
33
22
11
0
Reactivation index (%)
*
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP

Figure 4.

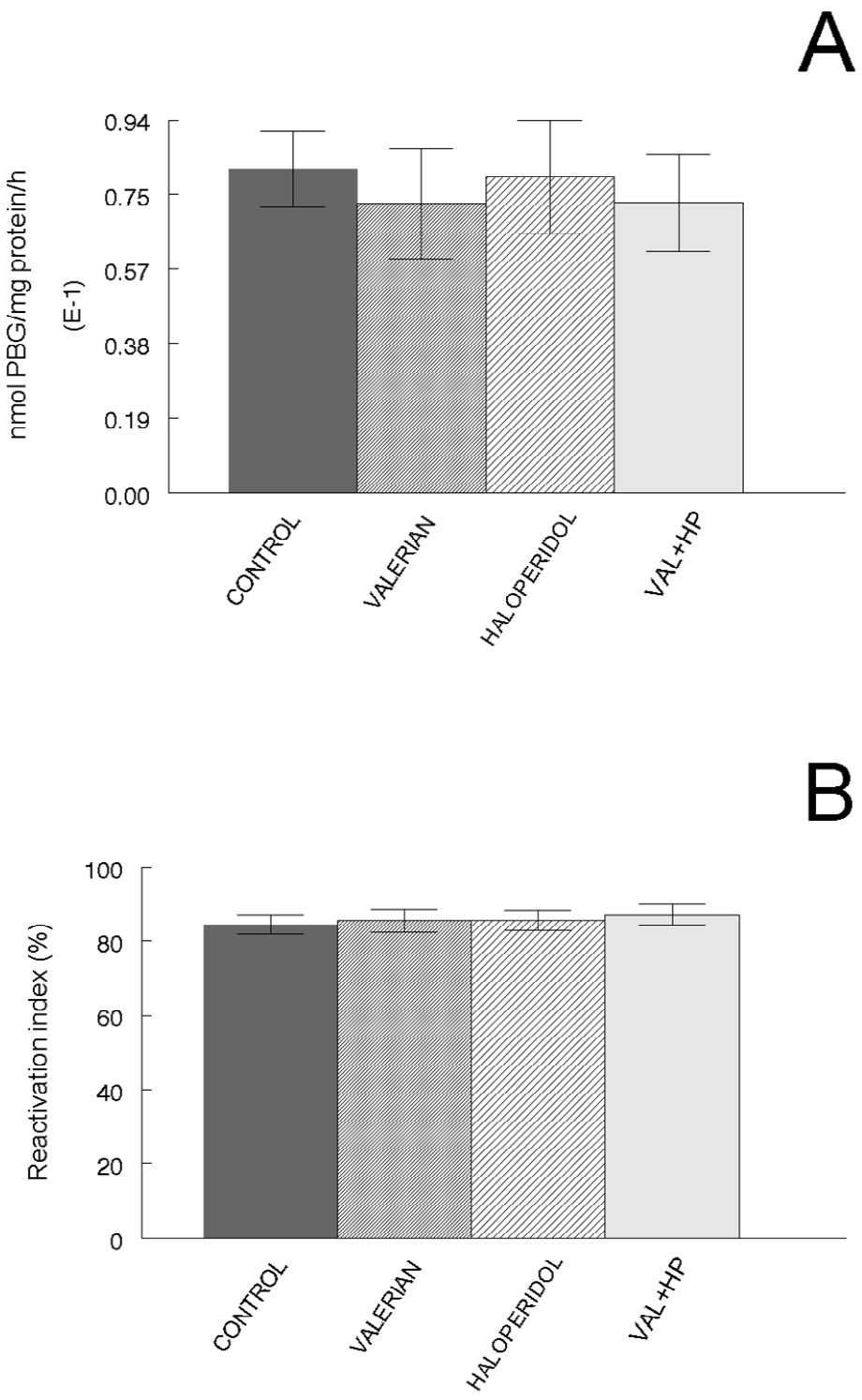

A
nmol PBG/mg protein/h
(E-1)
0.94
0.75
0.57
0.38
0.19
0.00
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP
B
Reactivation index (%)
100
80
60
40
20
0
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP

Figure 5.

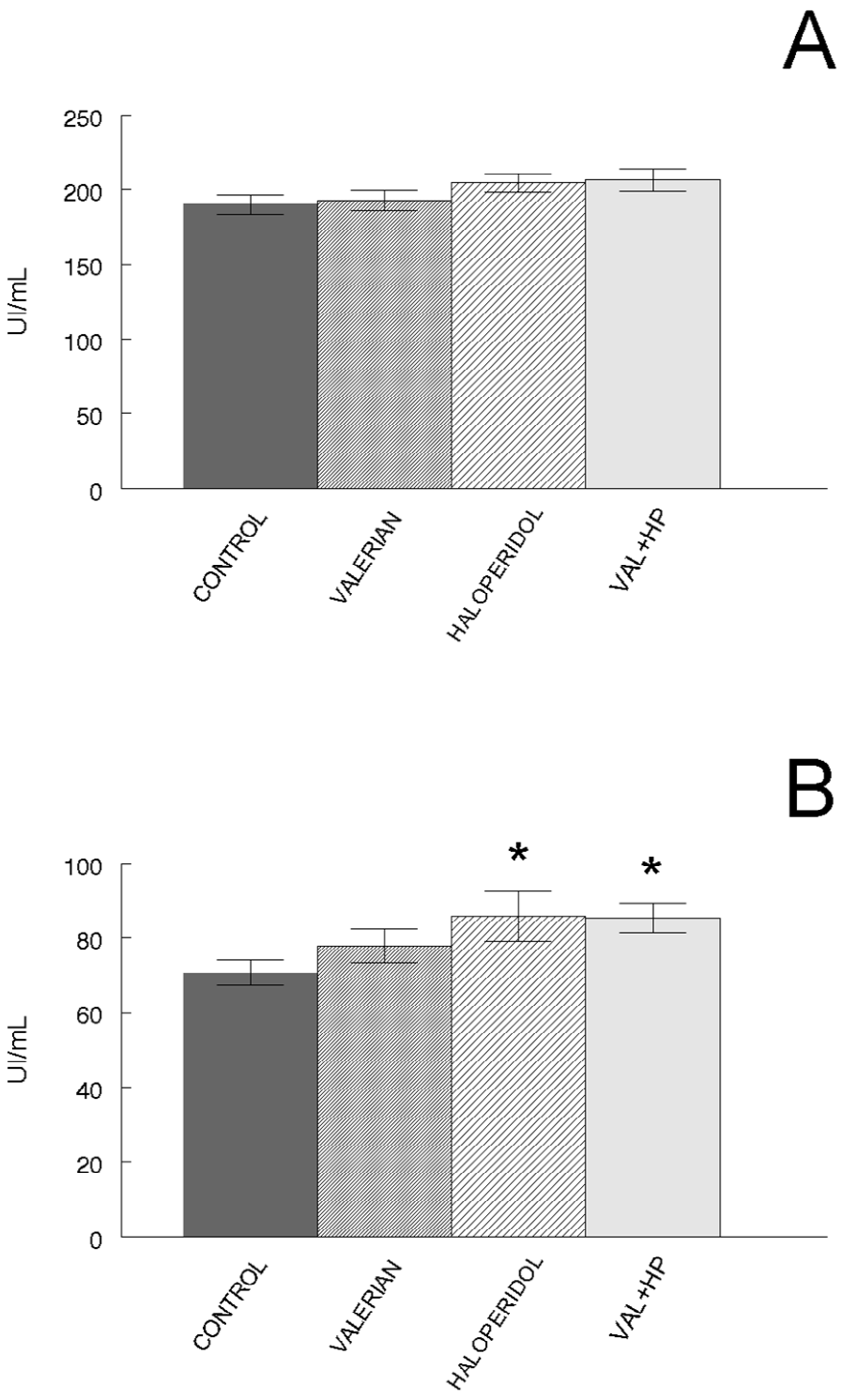
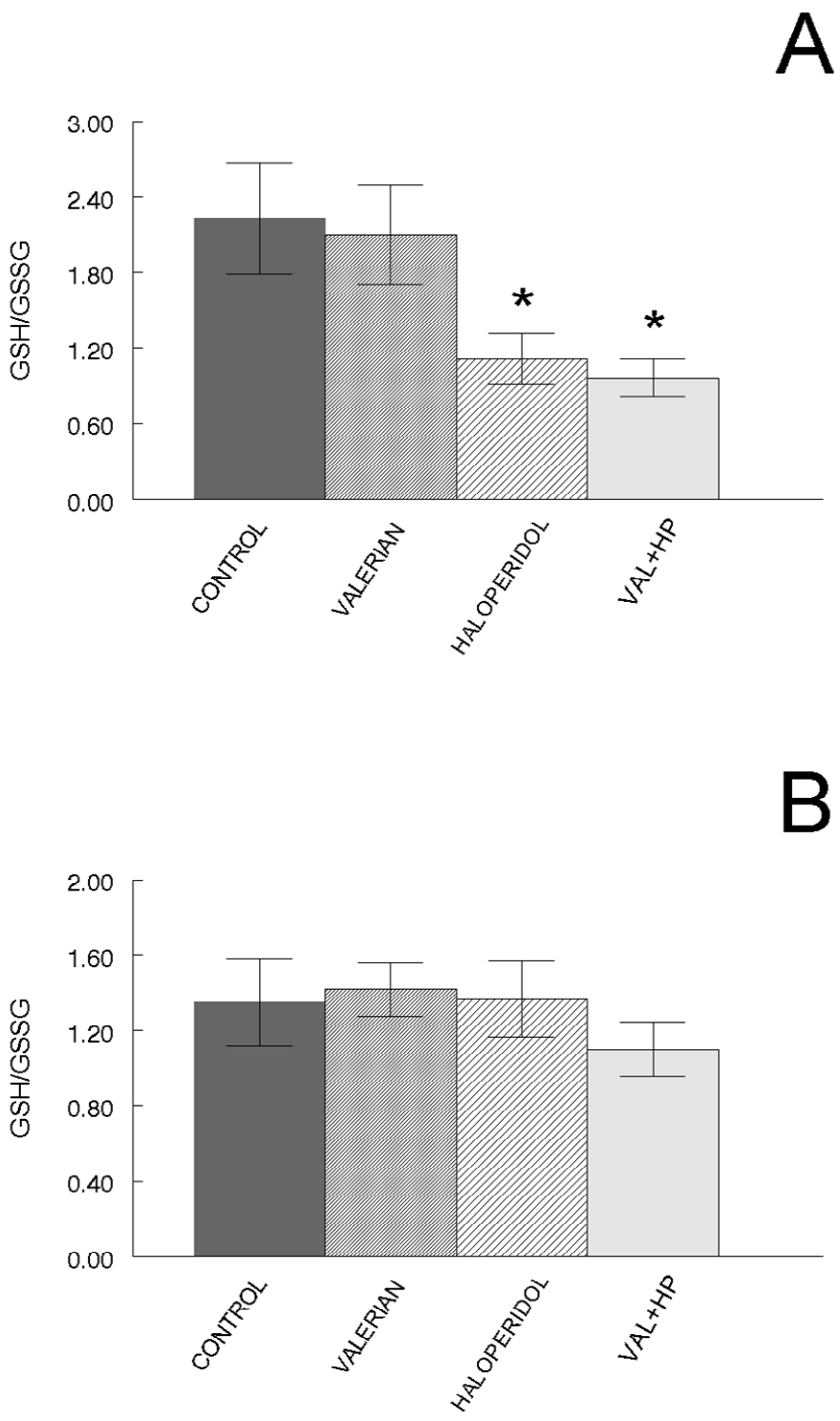

A
250
200
150
100
50
0
UI/mL
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP
B
100
80
60
40
20
0
UI/mL
*
*
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP

Figure 6.


A
3.00
2.40
1.80
1.20
0.60
0.00
GSH/GSSG
*
*
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP
B
2.00
1.60
1.20
0.80
0.40
0.00
GSH/GSSG
CONTROL
VALERIAN
HALOPERIDOL
VAL+HP

4. DISCUSSÃO

O uso de drogas neurolépticas tem sido associado a efeitos colaterais como a DT e o dano hepático. Apesar dos inúmeros casos de hepatotoxicidade após a administração de neurolépticos, são escassos os dados na literatura a respeito desses efeitos e o mecanismo exato pelo qual neurolépticos induzem hepatotoxicidade permanece incerto (Dumortier, 2002).

Um dos objetivos do presente estudo foi avaliar os efeitos do tratamento crônico com flufenazina sobre parâmetros de estresse oxidativo em fígado e rim de ratos bem como o possível efeito protetor do disseleneto de difenila sobre o dano induzido por flufenazina (artigo 1). Demonstramos neste estudo que a exposição prolongada à flufenazina provocou um aumento na peroxidação lipídica (TBARS), uma diminuição na atividade da SOD e um aumento na atividade da CAT no fígado de ratos. Estes resultados indicam que a administração crônica de flufenazina pode estar associada ao estresse oxidativo. A flufenazina também induziu peroxidação lipídica no rim, no entanto as atividades da SOD e da CAT não foram afetadas neste órgão.

As drogas fenotiazínicas são extensivamente oxidadas no organismo formando radicais catiônicos (O'Brien, 1988; Yang e Kulkarni, 1997; Galati e cols., 2002; Tafazoli e O'Brien, 2005). Eghbal e cols. (2004) demonstraram que em pH fisiológico enzimas peroxidases catalisam a oxidação de fenotiazinas em radicais pró-oxidantes, os quais oxidam ascorbato, GSH e NADH bem como induzem estresse oxidativo e citotoxicidade quando incubados com hepatócitos. Dessa forma, podemos sugerir que a peroxidação lipídica induzida por flufenazina poderia ocorrer devido à produção de metabólitos da flufenazina catalisada por enzimas peroxidases.

O disseleneto de difenila foi utilizado neste estudo devido às suas propriedades antioxidante e hepatoprotetora já descritas (Nogueira e cols., 2004; Borges e cols., 2005; 2006). Os resultados deste estudo demonstraram que o disseleneto de difenila foi eficiente em proteger o fígado e o rim da peroxidação lipídica (TBARS) induzida por flufenazina. Este efeito protetor do disseleneto de difenila sobre o TBARS foi acompanhado pela restauração parcial da atividade da CAT no fígado. O disseleneto de

difenila também foi capaz de restaurar a atividade da SOD no fígado dos ratos tratados com flufenazina. A diminuição na atividade da SOD causada pelo tratamento com flufenazina poderia contribuir para o aumento nos níveis de TBARS observados no fígado. Podemos, então, atribuir a proteção do disseleneto de difenila à sua atividade tiol-peroxidase e a outras propriedades antioxidantes deste composto (Arteel e cols., 2001; Rossato e cols., 2002; Nogueira e cols., 2004). Por outro lado, a atividade da SOD no rim foi diminuída somente quando a flufenazina foi administrada concomitantemente com o disseleneto de difenila. Este resultado foi inesperado, e pode indicar uma interação complexa entre as propriedades antioxidantes do composto de selênio e a diminuição em uma enzima antioxidante importante no rim. Apesar disso, a administração de disseleneto de difenila não foi acompanhada por nenhum sinal de peroxidação lipídica (TBARS) no rim.

O tratamento com flufenazina provocou uma inibição na atividade da δ-ALA-D e a associação da flufenazina com o disseleneto de difenila não foi capaz de restaurar a atividade da enzima. Esta associação aumentou a inibição parcial causada pela flufenazina sozinha. A flufenazina e o disseleneto de difenila poderiam estar interagindo de tal forma que a oxidação dos grupos sulfidrila (-SH) da δ-ALA-D foi mais pronunciada do que no grupo tratado apenas com a flufenazina. Estes dados são reforçados pelo fato do DTT, um agente redutor clássico, ter restaurado a inibição da δ-ALA-D (Perottoni e cols., 2005) e pelo índice de reativação, que foi o mais elevado no grupo tratado com flufenazina e disseleneto de difenila.

Ao contrário do fígado, a atividade da δ-ALA-D renal não foi alterada pelo tratamento com flufenazina, no entanto a associação de flufenazina com disseleneto de difenila resultou em inibição da atividade da enzima. O DTT foi capaz de restaurar a atividade da δ-ALA-D renal. Contudo, não houve diferença no índice de reativação para a δ-ALA-D renal entre os grupos. Com base neste resultado podemos sugerir que a ação inibitória dessas drogas sobre a atividade da δ-ALA-D no rim não está relacionada com a oxidação de grupos –SH. A redução da atividade da δ-ALA-D renal poderia ser

atribuída a um efeito aditivo do disseleneto de difenila e da flufenazina sobre a atividade da enzima.

O tratamento realizado neste trabalho foi baseado em estudos prévios, sendo um modelo de DO em roedores (See e cols., 1992; Van Kampen e Stoessl, 2000; Fachinetto e cols., 2007). Diversos autores já apresentaram evidências do envolvimento de EROS no desenvolvimento da DT (Cadet e cols., 1986; Lohr e cols., 1988; Burger e cols., 2003; Abílio e cols., 2004). Os resultados apresentados neste estudo podem contribuir para uma melhor compreensão dos efeitos colaterais relacionados ao uso de neurolépticos, pois, se um metabólito reativo é capaz de induzir estresse oxidativo no fígado o mesmo também poderia ser produzido no cérebro e, portanto, exercer efeitos deletérios neste tecido. Além disso, podemos supor que mesmo uma hepatotoxicidade limitada dos neurolépticos poderia facilitar a sua neurotoxicidade particularmente por aumentar a susceptibilidade do organismo como um todo aos efeitos danosos dos radicais livres.

Juntos os resultados deste primeiro artigo, contribuem para um maior entendimento dos danos hepáticos induzidos pela flufenazina. Os dados apresentados aqui indicam claramente o importante papel das EROS no tratamento crônico com flufenazina. Além disso, a proteção do disseleneto de difenila sobre o estresse oxidativo causado pela flufenazina nos dá uma indicação dos benefícios do disseleneto de difenila para proteger o fígado de uma variedade de agentes hepatotóxicos.

O segundo objetivo deste estudo foi o de avaliar os efeitos do tratamento crônico com haloperidol e *Valeriana officinalis* bem como a sua combinação sobre o fígado e o rim de ratos (artigo 2). Os resultados apresentados aqui demonstram que o haloperidol apenas alterou um parâmetro de estresse oxidativo no fígado aumentando a depleção de GSH, enquanto nenhum parâmetro foi alterado no rim. Por outro lado quando o haloperidol foi associado a valeriana houve um aumento significativo nos níveis de TBARS e na produção de espécies reativas (DCFH) no fígado. Nossos resultados indicam um aumento do dano oxidativo provocado pela associação de haloperidol e valeriana.

Apesar do dano oxidativo observado, as atividades das enzimas SOD e CAT não foram afetadas por nenhum dos tratamentos no fígado. Da mesma forma, o haloperidol não alterou as atividades da SOD e CAT plasmáticas em pacientes sob esta medicação (Yao e cols., 1998). O haloperidol é extensivamente metabolizado no fígado, e apenas 40 % da dose administrada é excretado na urina, sendo aproximadamente 1% excretado na forma inalterada (Forsman e cols., 1977). Dessa forma, não é surpresa que este tratamento não tenha causado nenhum efeito deletério sobre o rim de ratos neste estudo.

A *Valeriana officinalis* é largamente utilizada devido às suas propriedades sedativas, no entanto existe a necessidade de estudos sobre a segurança do uso prolongado de valeriana quando associada a outras drogas (Hadley e Petry, 2003). Neste estudo o tratamento com valeriana não alterou os níveis de TBARS, a oxidação da DCFH, e as atividades da SOD e da CAT. Al-Majed e colaboradores (2006) demonstraram o aumento nos níveis de MDA e uma diminuição nos níveis de –SH não protéico em células hepáticas de camundongos após tratamento sub-agudo com valeriana. Entretanto, no presente estudo apenas observou-se efeitos colaterais quando a valeriana foi associada ao haloperidol.

A atividade da δ-ALA-D foi avaliada por esta ser uma enzima que contém grupos SH os quais são suscetíveis a agentes oxidantes e, portanto, a enzima pode ser inibida após a sua exposição a situações pró-oxidantes (Folmer e cols., 2002, 2003; Soares e cols., 2003; Gonçalves e cols., 2005; Santos e cols., 2005). Haloperidol e valeriana quando administrados separadamente não afetaram a atividade da enzima, no entanto a sua associação resultou na perda de atividade da δ-ALA-D hepática. Haloperidol e valeriana poderiam estar interagindo de forma a oxidar os grupos –SH da δ-ALA-D provavelmente por produzirem algum metabólito mais tóxico para enzima. Este resultado é reforçado pela restauração da atividade da δ-ALA-D pelo DTT (Perottoni e cols., 2005), e pelo índice de reativação para a δ-ALA-D o qual foi mais alto no tratamento combinado que nos outros grupos tratados. Este resultado corrobora a hipótese de que a enzima foi inibida pela oxidação dos seus grupos -SH. Foi relatado que o valtrato, um componente da valeriana, pode interagir com grupos –SH

(Keochanthala-Bounthanh e cols., 1990). Talvez o haloperidol possa aumentar esta capacidade da valeriana de interagir com grupos –SH ou afetar a suscetibilidade do fígado. Estes resultados fortalecem a visão de que a δ-ALA-D pode ser considerada um marcador de estresse oxidativo.

A liberação de enzimas intracelulares (AST e ALT) é um indicativo de dano às membranas celulares e é utilizado como um índice de hepatotoxicidade (Cohen e Kaplan, 1979). A ALT é uma enzima citoplasmática e encontra-se principalmente no fígado, enquanto a AST é predominantemente mitocondrial e pode aparecer elevada em doenças de outros órgãos, como o coração ou o músculo. Dessa forma, elevações na ALT representam um marcador mais sensível de função hepática (Motta, 2003). A atividade da AST no soro não foi alterada por nenhum dos tratamentos. No entanto, a atividade da ALT no soro foi maior nos ratos tratados com haloperidol e com o tratamento combinado. Gaertner e colaboradores (2001) reportaram uma elevação na atividade das transaminases em pacientes tratados com haloperidol, o que corrobora estes resultados. Contudo, o aumento na atividade da ALT observado neste estudo não foi alto o suficiente para representar uma preocupação clínica. Aumentos de 3 a 50 vezes na atividade das transaminases no soro são encontrados frequentemente em doenças hepáticas (Motta, 2003).

Neste estudo o haloperidol causou uma diminuição na razão GSH/GSSG no fígado, o que também foi observado no tratamento combinado com haloperidol e valeriana. A razão GSH/GSSG indica o balanço entre antioxidantes e oxidantes. Sendo assim, um aumento nos níveis de GSSG e uma redução na razão GSH/GSSG é um indicativo de estresse oxidativo (Jones e cols., 2000). O aumento na depleção de GSH prejudica as defesas celulares e poderia estar contribuindo para o aumento no TBARS e para a oxidação da DCFH.

Os resultados apresentados aqui apontam para uma interação entre o haloperidol e a valeriana no aumento do estresse oxidativo no fígado. Gold e colaboradores (2001) identificaram uma série de interações em potencial entre produtos naturais, incluindo valeriana, e terapias com drogas convencionais. Interações plantas medicinais-fármacos

podem aparecer quando plantas medicinais e fármacos são administrados concomitantemente e o preparado da planta (um ou mais componentes) modula o metabolismo da droga pela indução ou inibição das enzimas citocromo P450. Hellum e colaboradores (2006) mostraram a capacidade dos extratos de valeriana em induzir as enzimas CYP em culturas primárias de hepatócitos humanos, incluindo CYP3A4, a mais importante das enzimas P450 responsáveis pela via do metabolismo do haloperidol (Igarashi e cols., 1995; Usuki e cols., 1996; Fang e cols., 2001; Kalgutkar e cols., 2003; Avent e cols., 2006). Com base nesses trabalhos, nós supomos que o mecanismo pelo qual a associação de valeriana e haloperidol induz dano oxidativo pode estar relacionado com a atividade da CYP. Os efeitos indutores da valeriana sobre as enzimas CYP poderiam aumentar a produção de metabólitos tóxicos do haloperidol como o HPP^{+}, ocasionando os efeitos deletérios observados no tratamento com haloperidol e valeriana no fígado. Estes efeitos adversos do tratamento com valeriana e haloperidol no fígado e a ausência destes efeitos no rim também poderiam ser explicados por deste mecanismo. As enzimas CYP são encontradas nos níveis mais altos no fígado, e expressa em baixos níveis no rim (Gonzalez, 2005). O fígado, dessa forma, seria o principal sítio de interação erva-droga onde a produção de metabólitos tóxicos poderia estar elevada.

As doses de valeriana e haloperidol utilizadas neste estudo estão dentro de uma faixa normal para humanos, mas uma possível interação negativa entre estas duas drogas irá ocorrer em uma situação real em humanos apenas após a ingestão de doses supraterapêuticas de valeriana e haloperidol. De qualquer modo, um possível efeito aditivo tóxico destes dois compostos deve ser considerado. Dessa forma, a administração de valeriana em pessoas que estejam sob medicação com antipsicóticos deve ser melhor estudada para evitar possíveis interações negativas.

O uso de plantas medicinais como terapia alternativa e/ ou complementar nos países ocidentais vem ganhando grande popularidade (Hellum e cols., 2006). Infelizmente, o seu uso tem sido acompanhado pela crença de que se é natural é igualmente seguro. O uso difundido de suplementos de valeriana indica que o seu uso com medicamentos convencionais é perfeitamente possível. Além disso, o potencial

para interações de drogas com produtos naturais ainda permanece bastante indefinido (Fugh-Berman e Ernst, 2001). As descobertas deste estudo sugerem efeitos adversos nas interações entre valeriana e haloperidol causando dano hepático relacionado ao estresse oxidativo. Estudos posteriores são necessários para elucidar o exato mecanismo pelo qual os compostos testados aqui causam estresse oxidativo. Além disso, é necessário cautela ao tentar extrapolar estes resultados para outras plantas medicinais e fármacos.

5. CONCLUSÕES

Neste trabalho salientamos a importância da vigilância cuidadosa dos efeitos colaterais de drogas neurolépticas especialmente sobre a função hepática. Os resultados apresentados nesta dissertação indicam a possibilidade de um dano oxidativo induzido pela flufenazina no fígado e no rim de ratos. Além disso, o disseleneto de difenila demonstrou-se eficaz em proteger contra o dano oxidativo causado pela flufenazina no tecido hepático. De acordo com estes dados podemos concluir que a administração de flufenazina esta associada à produção de EROS. Além disso, podemos sugerir que o estresse oxidativo poderia ser um mecanismo para explicar a hepatotoxicidade provocada pelo tratamento com flufenazina.

A partir deste estudo também podemos concluir que a administração de haloperidol e *V. officinalis* separadamente não parece apresentar nenhum risco às funções hepáticas e renais em ratos. No entanto, a combinação de haloperidol e valeriana provocou um dano oxidativo no fígado de ratos, talvez pela indução de enzimas CYP e aumento na produção de metabólitos tóxicos do haloperidol. Dessa forma, o uso de valeriana não seria recomendado a pacientes que estejam sob tratamento com haloperidol.

6. REFERÊNCIAS BIBLIOGRÁFICAS

ABÍLIO, V.C., SILVA, R.H., CARVALHO, R.C., GRASSL, C., CALZAVARA, M.B., REGISTRO, S., D'ALMEIDA, V., RIBEIRO, R.A., FRUSSA-FILHO, R. Important role of striatal catalase in aging- and reserpine-induced oral dyskinesia. Neuropharmacology, 47, 263-272, 2004.

AL-MAJED, A.A., AL-YAHYA, A.A., AL-BEKAIRI, A.M., AL-SHABANAH, O.A., QURESHI, S. Studies on the cytological and biochemical effects of valerian in somatic and germ cells of Swiss albino mice. Food Chem Toxicol., 44, 1830-1837, 2006.

ANDREASSEN, O.A., JØRGENSEN H.A. Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats Implications for tardive dyskinesia? Prog Neurobiol., 61, 525-541, 2000.

ARTEEL, G.E., SIES, H. The biochemistry of selenium and glutathione system. Environ Toxicol Pharmacol., 10, 153-158, 2001.

AVENT, K.M., DEVOSS, J.J., GILLAM, E.M. Cytochrome P450-mediated metabolism of haloperidol and reduced haloperidol to pyridinium metabolites. Chem Res Toxicol., 19, 914-920, 2006.

BARBOSA, N.B.V., ROCHA, J.B.T., ZENI, G., EMANUELLI, T., BEQUE, M.C., BRAGA, A.L. Effect of organic forms of selenium on (-aminolevulinate dehydratase from liver, kidney and brain of adult rats. Toxicol Appl Pharmacol., 49, 243-253, 1998.

BECHARA, E.J.H, MEDEIROS, M.H.G., MONTEIRO, H.P., HERMES-LIMA, M., PEREIRA, B., DEMASI, M., COSTA, C.A., ADBALLA, D.S.P., ONUKI, J., WENDEL, C.M.A., MASCI, P.D. A free radical hypothesis of lead poisoning and in Born porphyrias associated with 5-aminolevulinic overload. Quim Nova., 16, 385-392, 1993.

BECHARA, E.J.H. Oxidative stress in acute intermittent porphyria and lead poisoning may be triggered by 5-aminolevulinic acid. Braz J M Biol Res., 29, 841-851, 1996.

BEHL, C., RUPPRECHT, R., SKUTELLA, T., HOLSBOER, F. Haloperidol-induced cell death-mechanism and protection with vitamin E in vitro. Neuroreport, 7, 360–364, 1995.

BEHL, C., LEZOULAC'H, F., WIDMANN, M., RUPPRECHT, R., HOLSBOER, F. Oxidative stress-resistant cells are protected against haloperidol toxicity. Brain Res., 717, 193–195, 1996.

BLUMENTHAL, M. Herbs continue slides in mainstream market: sales down 14 percent. HerbalGram., 58, 71, 2003.

BORGES, L.P., BORGES, V.C., MORO, A.V., NOGUEIRA, C.W., ROCHA, J.B.T., ZENI, G. Protective effect of diphenyl diselenide on acute liver damage induced by 2-nitropropane in rats. Toxicology, 210, 1–8, 2005.

BORGES, L.P., NOGUEIRA, C.W., PANATIERI, R.B., ROCHA, J.B.T., ZENI, G. Acute liver damage induced by 2-nitropropane in rats: Effect of diphenyl diselenide on antioxidant defenses. Chem Biol Interac., 160, 99–107, 2006.

BURGER, M.E., ALVES, A., CALLEGARI, L., ATHAYDE, F.R., NOGUEIRA, C.W., ZENI, G., ROCHA, J.B.T. Ebselen attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum. Prog Neuro-psychopharmacol., 27, 135-140, 2003.

BURGER, M., FACHINETTO, R., CALEGARI, L., PAIXÃO, M.W., BRAGA, A.L., ROCHA, J.B. Effects of age on reserpine-induced orofacial dyskinesia and possible protection of diphenyl diselenide. Brain Res Bull., 64, 339-345, 2004.

BURGER, M.E., FACHINETTO, R., ZENI, G., ROCHA, J.B. Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain. Pharmacol Biochem Behav., 81, 608-615, 2005.

BURGER, M.B., FACHINETTO, R., WAGNER, C., PEROTTONI, J., PEREIRA, R.P., ZENI, G., ROCHA, J.B.T. Effects of diphenyl diselenide on orofacial dyskinesia model in rats. Brain Res Bull., 70, 165–170, 2006.

BURKHARDT, C., KELLY, J.P., LIM, Y.H., FILLEY, C.M., PARKER JR, W.D. Neuroleptic medications inhibit complex I of the electron transport chain. Ann Neurol., 33, 512–517, 1993.

BURT, D., CREESE, I., SNYDER, S.H. Antishizophrenic drugs: Chronic treatment elevates dopamine receptor binding in brain. Science, 196, 326–327, 1977.

CADET, J.L., LOHR, J.B., JESTE, D.V. Free radical and tardive dyskinesia. Trends Neurosci., 9, 107-108, 1986.

CASEY, D.E. Tardive dyskinesia: reversible and irreversible. Psychopharmacology, 2, 88-97, 1985.

CAVADAS, C., ARAUJO I., COTRIM, M.D., AMARAL, T., CUNHA, A.P., MACEDO, T., RIBEIRO, C.F. In vitro study on the interaction of Valeriana officinalis L. extracts and their amino acids on GABAA receptor in rat brain. Arzneimittelforschung, 45, 753-755, 1995.

CAVALLARO, R., SMERALDI, E. Antipsychotic-induced tardive dyskinesia: recognition, prevention and management, CNS Drugs, 4, 278–293, 1995.

CHAN, T.Y., TANG, C.H., CRITCHLEY, J.A. Poisoning due to an over-the-counter hypnotic, Sleep-Qik (hyoscine, cyproheptadine, valerian). Postgrad Med J., 71, 227-228, 1995.

CHAN, T.Y.K. An assessment of the delayed effects associated with valerian overdose. Int J Clin Pharmacol Ther., 36, 569, 1998.

COHEN, J.A., KAPLAN, M.M. The SGOT/SGPT ratio--an indicator of alcoholic liver disease. Dig Dis Sci., 24, 835-838, 1979.

CRANE, G.E. Persistent dyskinesia. Br J Psychiatry, 122, 395-405, 1973.

CREESE, I., BURT, D.R., SNYDER, S.H. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science, 192, 481–483, 1976.

DE KEYSER, K. Excitotoxic mechanisms may be involved in the pathophysiology of tardive dyskinesia. Clin Neuropharmacol., 14, 562–565, 1991.

DELEVE, L., WANG, X., KUHLENKAMP, J., KAPLOWITZ, N. Toxicity of azathioprine and monocrotaline in murine sinusoidal endothelial cells and hepatocytes: the role of glutathione and relevance to hepatic venooclusive disease. Hepatology, 23, 589–599, 1996.

DIETZ, B.M., MAHADY, G.B., PAULI, G.F., FARNSWORTH, N.R. Valerian extract and valerenic acid are partial agonists of the 5-HT_{5a} receptor in vitro. Mol Brain Res., 138, 191-197, 2005.

DUMORTIER, G., CABARET, W., STAMATIADIS, L., SABA, G., BENADHIRA, R., ROCAMORA, J.F., AUBRIOT-DELMAS, B., GLIKMAN, J., JANUEL, D. Hepatic tolerance of atypical antipsychotic drugs. Encephale, 28, 542-551, 2002.

EGAN, M.F., APUD, J., WYATT, R.J. Treatment of tardive dyskinesia. Schiz Bull., 23, 583–609, 1997.

EGHBAL, M.A., TAFAZOLI, S., PENNEFATHER, P., O'BRIEN, P.J. Peroxidase catalysed formation of cytotoxic prooxidant phenothiazine free radicals at physiological pH. Chem Biol Interac., 151, 43-51, 2004.

EISENBERG, D.M., DAVIS, R.B., ETTNER, S.L., APPEL, S., WILKEY, S., ROMPAY, M.V., KESSLER, R. Trends in alternative medicine use in United States, 1990-1997: results of a follow-up national survey. JAMA, 280, 1569-1575, 1998.

FACHINETTO, R., VILLARINHO, J.G., WAGNER, C., PEREIRA, R.P., ÁVILA, D.S., BURGER, M.E., CALIXTO, J.B., ROCHA, J.B., FERREIRA, J. Valeriana officinalis does not alter the orofacial dyskinesia induced by haloperidol in rats: role of dopamine transporter. Prog NeuroPsychopharmacol Biol Psychiatry, 31, 1478-1486, 2007.

FANG, J., MCKAY, G., SONG, J., REMILLRD, A., LI, X., MIDHA, K. In vitro characterization of the metabolism of haloperidol using recombinant cytochrome P450 enzymes and human liver microsomes. Drug Metab Dispos., 12, 1638–1643, 2001.

FARINA, M., BARBOSA, N.B.V., NOGUEIRA, C.W., FOLMER, V., ZENI, G., ANDRADE, L.H., BRAGA, A.L., ROCHA, J.B.T. Reaction of diphenyl diselenide with hydrogen peroxide and inhibition of delta-aminolevulinate dehydratase from rat liver and cucumber leaves. Braz J Med Biol Res., 35, 623-631, 2002.

FIBIGER, H.C., LLOYD, K.G. Neurobiological substrates of tardive dyskinesia: the GABA hypothesis. Trends Neurosci., 7, 462–464, 1984.

FOLMER, V., SOARES, J.C., ROCHA, J.B.T. Oxidative stress in mice is dependent on the free glucose content of the diet. Int J Biochem Cell Biol., 34, 1279-1285, 2002.

FOLMER, V., SOARES, J.C., GABRIEL, D., ROCHA, J.B.T. A high fat diet inhibits delta-aminolevulinate dehydratase and increases lipid peroxidation in mice (Mus musculus). J Nutr., 133, 2165-2170, 2003.

FORSMAN, A., FOLSCH, G., LARSSON, M., OHMAN, R. On the metabolism of haloperidol in man. Curr Ther Res., 21, 606-617, 1977.

FUGH-BERMAN, A., ERNST, E. Herb-drug interactions: review and assessment of report reliability. Br J Clin Pharmacol., 52, 587-595, 2001.

GAERTNER, I., ALTENDORF, K., BATRA, A., GAERTNER, H.J. Relevance of liver enzyme elevations with four different neuroleptics: a retrospective review of 7,263 treatment courses. J Clin Psychopharmacol., 21, 215-222, 2001.

GALATI, G., TAFAZOLI, S., SABZEVARI, O., CHAN, T.S., O'BRIEN, P.J. Idiosyncratic NSAID drug induced oxidative stress. Chem Biol Interact., 142, 25–41, 2002.

GAO, X.M., KAKIGI, T., FRIEDMAN, M.B., TAMMINGA, C.A. Tiagabine inhibits haloperidol-induced oral dyskinesias in rats. J Neural Transm Gen Sect., 95, 63–69, 1994.

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

GLAZER, M.N., MORGENSTERN, H., SCHOOLER, N., BERKMAN, C.S., MOORE, D.C. Predictors of improvement in tardive dyskinesia following discontinuation of neuroleptic medication. Br J Psychiatry, 157, 585-592, 1990.

GOLD, J.L., LAXER, D.A., DERGAL, J.M., LANCTOT, K.L., ROCHON, P.A. Herbal-drug therapy interaction: a focus on dementia. Curr Opin Clin Nutr Metab Care, 4, 29-34, 2001.

GONÇALVES, T.L., ERTHAL, F., CORTE, C.L.D., MULLER, L.G., PIOVEZAN, C.M., NOGUEIRA, C.W., ROCHA, J.B.T. Involvement of oxidative stress in the pre-malignant and malignant states of cervical cancer in women. Clin Biochem., 38, 1071-1075, 2005.

GONZALEZ, F.J. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. Mutat Res., 569, 101-110, 2005.

GOODMAN, LOUIS S. Goodman & Gilman, As Bases Farmacologicas da Terapeutica (10 ed.) Rio de Janeiro, Mcgraw-Hill, 2004.

GOPAUL, S., FRREL, K., ABBOUT, F. Effects of age and politherapy risk factors of valproic acid (VPA) hepatotoxicity on the excretion of thiol conjugates of 4-diene-VPA in people with epilepsy taking VPA. Epilepsia, 44, 322-328, 2003.

GUNNE, L.M., HAGGSTROM, J.E. Reduction of nigral glutamic acid decarboxylase in rats with neuroleptic-induced oral dyskinesia. Psychopharmacology, 81, 191–194, 1983.

GUNNE, L.M., HAGGSTROM, J.E., SJOQUIST, B. Association with persistent neuroleptic-induced dyskinesia of regional changes in brain GABA synthesis. Nature, 309, 347–349, 1984.

GUTIERREZ, S., ANG-LEE, M.K., WALKER, D.J., ZACNY, J.P. Assessing subjective and psychomotor effects of the herbal medication valerian in healthy volunteers. Pharmacol Biochem Behav., 78, 57-64, 2004.

HADLEY, S., PETRY, J.J. Valerian. Am Fam Physician, 67, 1755-1758, 2003.

HALLER, C.A., DYER, J.E., KO, R., OLSON, K.R. Making a diagnosis of herbal-related toxic hepatitis. West J Med., 176, 39-44, 2002.

HALLIWELL, B., GUTTERIDGE, J. M.C. Free radical in biology and medicine (3rd.) Oxford University Press, 1999.

HELLUM, B.H., HU, Z., NILSEN, O.G. The Induction of CYP1A2, CYP2D6 and CYP3A4 by six trade herbal products in cultured primary human hepatocytes. Basic Clin Pharmacol Toxicol., 100, 23–30, 2006.

HORROBIN, D.F., MANKU, M.S., MORSE-FISHER, N., VADDADI, K.S., COURTNEY, P., GLEN, A.I., GLEN, E., SPELLMAN, M., BATES, C. Essential

fatty acids in plasma phospholipids in schizophrenics. Biol Psychiatry., 25, 562-568, 1989.

HOUGHTON, P. The scientific basis for the reputed activity of Valerian. J Pharm Pharmacol., 51, 505-12, 1999.

HUI-LIAN, W., DONG-FANG, Z., ZHAO-FENG, L., YANG, L., QIAN-RONG, L., YU-ZHEN, W. In vitro study on the genotoxicity of dichloromethane extracts of valerian (DEV) in human endothelial ECV304 cells and the effect of vitamins E and C in attenuating the DEV-induced DNA damages. Toxicol Appl Pharmacol., 188, 36–41, 2003.

IGARASHI, K., KASUYA, F., FUKUI, M., USUKI, E., CASTAGNOLI JR., N. Studies on the metabolism of haloperidol (HP): the role of CYP3A in the production of the neurotoxic pyridinium metabolite HPP^+ found in rat brain following IP administration of HP. Life Sci., 57, 2439-2446, 1995.

JACKSON-LEWIS, V., PRZEDBORSKI, S., KOSTIC, V., SUBER, F., FAHN, S., CADET, J.L. Partial attenuation of chronic fluphenazine-induced changes in regional monoamine metabolism by D-alpha-tocopherol in rat brain. Brain Res Bull., 26, 251–258, 1991.

JESTE, D.V., POTKIN, S.G., SINHA, S., FEDER, S., WYATT, R.J. Tardive dyskinesia-reversible and persistent. Arch Gen Psychiatry, 36, 585-590, 1979.

JOHANSSON, P.E., TERENIUS, L., HAGGSTROM, J.E., GUNNE, L. Neuropeptide changes in a primate model (Cebus apella) for tardive dyskinesia. Neuroscience, 37, 563–567, 1990.

JONES, D.P., CARLSON, J.L., MODY, V.C., CAI, J., LYNN, M.J., STERNBERG, P. Redox state of glutathione in human plasma. Free Radic Biol Med., 28, 625-635, 2000.

KALGUTKAR, A.S., TAYLOR, T.J., VENKATAKRISHNAN, K., ISIN, E.M. Assesment of the contributions of CYP3A4 and CYP3A5 in the metabolism of the antpsychotic agent haloperidol to its potencially neurotoxin pyridinium metabolite and effect of antidepressants on the bioactivation pathway. Drug Metab Dispos., 31, 243-249, 2003.

KANE, J.M., SMITH, J.M. Tardive dyskinesia: prevalence and risk factors, 1959 to 1979. Arch Gen Psychiatry, 39, 473–481, 1982.

KANE, J.M. Tardive dyskinesia: epidemiology and clinical presentation. In: BLOOM, F.E.; KUPFER, D.J. (Ed.) Psychopharmacology: the fourth generation of progress, vol. 39. Raven Press, New York, pp. 1485–1495, 1995.

KANEDA, H., SHIRAKAWA, O., DALE, J., GOODMAN, L., BACHUS, S.E., TAMMINGA, C.A. Co-administration of progabide inhibits haloperidol-induced oral dyskinesias in rats. Eur J Pharmacol., 212, 43–49, 1992.

KAPLOWITZ, N. Drug metabolism and hepatotoxicity. In: Kaplowitz, N. (Ed.) Liver and biliary diseases. 2nd ed. Baltimore: Williams & Wilkins, pp. 103–20, 1996.

KAPLOWITZ, N. Cell death at the millennium: implications for liver distress. Clin Liver Dis., 4, 1–23, 2000.

KAPLOWITZ, N. Biochemical and cellular mechanisms of toxic liver injury. Semin Liver Dis., 22, 137–144, 2002.

KEOCHANTHALA-BOUNTHANH, C., HAAG-BERRURIER, M., BECK, J.P., ANTON, R. Effects of thiol compounds versus the cytotoxicity of valepotriates on cultured hepatoma cells. Planta Med., 56, 190-192, 1990.

KLAWANS, H.L., RUBOVITS, R. An experimental model of tardive dyskinesia. J Neural Trans., 33, 235–246, 1972.

KLOTZ, L.O., SIES, H. Defenses against peroxynitrite: selenocompounds and flavonoids. Toxicol Lett., 14, 125–132, 2003.

KONO, H., ARTEEL, G.E., RUSYN, I., SIES, H., THURMAN, R.G. Ebselen prevents early alcohol-induced liver injury in rats. Free Rad Biol Med., 30, 403-411, 2001.

KONRADI, A.C., HECKERS, S. Molecular aspects of glutamate dysregulation: implications for schizophrenia and its treatment. Pharmacol Ther., 97, 153-179, 2003.

KOVACIC, P., JACINTHO, J.D. Mechanisms of carcinogenesis: Focus on oxidative stress and electron transfer. Curr Med Chem., 8, 773–796, 2001.

KOYANAGI, T., NAKAMUTA, M., ENJOJI, M., IWAMOTO, H., MOTOMURA, K., SAKAI, H., NAWATA, H. The selenoorganic compound ebselen suppresses liver

injury induced by Propionibacterium acnes and lipopolysaccharide in rats. Int J Mol Med., 7, 321-327, 2001.

KRYSTAL, A.D., RESSLER, I. The use of valerian in neuropsychiatry. CNS Spectr., 6, 841-847, 2001.

LEE, A. Drug-induced hepatotoxicity. N Engl J Med., 349, 474-85, 2003.

LEVEQUE, J.-C., MACÍAS, W., RAJADHYAKSHA, A., CARLSON, R.R., BARCZAK, A., KANG, S., LI, X.-M., COYLE, J.T., HUGANIR, R.L., HECKERS S., KONRADI, C. Intracellular modulation of NMDA receptor function by antipsychotic drugs. J Neurosci., 20, 4011–4020, 2000.

LEVINSON, D.F. Pharmacologic treatment of schizophrenia. Clin Ther., 13, 326–352, 1991.

LI, Q.-J., BESSEMS, J.G., COMMANDEUR, J.N., ADAMS, B., VERMEULEN, N.P. Mechanism of protection of ebselen against paracetamol-induced toxicity in rat hepatocytes. Biochem Pharmacol., 48, 1631-1640, 1994.

LOHR, J.B., CADET, J.L., LOHR, M.A., LARSON, L., WASLI, E., WADE, L. Vitamin E in the treatment of tardive dyskinesia: The possible involvement of free radical mechanisms. Schizophr Bull., 14, 291-296, 1988.

LOHR, J.B., KUCZENSKI, R., BRACHA, H.S., MOIR, M., JESTE, D.V. Increased indices of free radical activity in the cerebrospinal fluid of patients with tardive dyskinesia. Biol Psychiatry., 28, 535-539, 1990.

MAIORINO, M., ROVERI, A., COASSIN, M., URSINI, F. Kinetic mechanism and substrate specificity of glutathione peroxidase activity of ebselen (PZ51). Biochem Pharmacol., 37, 2267-2271, 1988.

MAIORINO, M., ROVERI, A., URSINI, F. Antioxidant effect of ebselen (PZ 51): Peroxidase mimetic activity on phospholipid and cholesterol hydroperoxides vs free radical scavenger activity. Arch Biochem Biophys., 295, 404-409, 1992.

MELTZER, H.Y., STAHL, S.M. The dopamine hypothesis of schizophrenia: a review. Schizophr Bull., 2, 19–76, 1976.

MENNINI, T., BERNASCONI, P., BOMBARDELLI, E., MORAZZONI, P. In vitro study on the interaction of extracts and pure compounds from Valeriana officinalis

roots with GABA, benzodiazepine and barbiturate receptors in rat brain. Fitoterapia, 64, 291–300, 1993.

MEOTTI, F.C., BORGES, V.C., ZENI, G., ROCHA, J.B.T., NOGUEIRA, C.W. Potential renal and hepatic toxicity of diphenyl diselenide, diphenyl ditelluride and ebselen for rats and mice. Toxicol Lett., 143, 9–16, 2003.

MILLER, D.M., BUETTNER, G.R., AUST, S.D. Transition metals as catalyst of ‘’autoxidation’’ reactions. Free Radic Biol Med., 8, 95-108, 1990.

MOTTA, V.T. Bioquímica Clínica para o Laboratório: princípios e interpretações. (4 ed.) Porto Alegre, Editora Médica Missau, 2003.

MUGESH, G., PANDA, A., SINGH, H.B., PUNEKAR, N.S., BUTCHER, R.J. Glutathione peroxidase-like antioxidant activity of diaryl diselenides: a mechanistic study. J Am Chem Soc., 123, 839-850, 2001.

MÜLLER, A., GABRIEL, H., SIES, H. A novel biologically active selenoorganic compound-IV. Protective glutathione-dependent effect of PZ51 (Ebselen) against ADP-Fe induced lipid peroxidation in isolated hepatocytes. Biochem Pharmacol., 34, 1185–1189, 1985.

NOGUEIRA, C.W., ROTTA, L.N., ZENI, G., SOUZA, D.O., ROCHA, J.B.T. Exposure to ebselen changes glutamate uptake and release by rat brain synaptosomes. Neurochem Res., 27, 283–288, 2002.

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, 2003a.

NOGUEIRA, C.W., QUINHONES, E.B., JUNG, E.A., ZENI, G., ROCHA, J.B. Anti-inflammatory and antinociceptive activity of diphenyl diselenide. Inflamm Res., 52, 56-63, 2003b.

NOGUEIRA, C.W., ZENI, G., ROCHA, J.B.T. Organoselenium and organotellurium compounds: Toxicology Pharmacology. Chem Rev., 104, 6255-6285, 2004.

NOVELLI, A., REILLY, J.A., LYSKO, P.G., HENNEBERRY, R.C. Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res., 451, 205–212, 1988.

O'BRIEN, P.J. Radical formation during the peroxidase catalyzed metabolism of carcinogens and xenobiotics: the reactivity of these radicals with GSH, DNA, and unsaturated lipid. Free Radic Biol Med., 4, 169–183, 1988.

ODIN, J.A., HUEBERT, R.C., CASCIOLA-ROSEN, L., LARUSSO, N.F., ROSEN, A. Bcl-2–dependant oxidation. J Clin Invest., 108, 223–232, 2001.

ORTIZ, J.G., NIEVES-NATAL, J., CHAVEZ, P. Effects of *Valeriana Officinalis* extracts on [^{3}H]Flunitrazepam binding, synaptosomal [^{3}H]GABA uptake, and hippocampal [^{3}H]GABA release. Neurochem Res., 24, 1373-1378, 2004.

OZAKI, M., NAKAMURA, M., TERAOKA, S., OTA, K. Ebselen, a novel anti-oxidant compound, protects the rat liver from ischemia-reperfusion injury. Transpl Int., 10, 96-102, 1997.

PARK, B.K., KITTERINGHAM, N.R., MAGGS, J.L., PIRMOHAMED, M., WILLIAMS, D.P. The role of metabolic activation in drug-induced hepatotoxicity. Annu Rev Pharmacol Toxicol., 45, 177–202, 2005.

PARNHAM, M.J., GRAF, E. Pharmacology of synthetic organic selenium compounds. Prog Drug Res., 36, 9-47, 1991.

PEROTTONI, J., MEOTTI, F.C., FOLMER, V., PIVETTA, L., NOGUEIRA, C.W., ZENI, G., ROCHA, J.B.T. Ebselen and diphenyl diselenide do not change the inhibitory effect of lead acetate on delta aminolevulinate dehydratase. Environ Toxicol Pharmacol., 19, 239-248, 2005.

REMINGTON, G., CHONG, S.A. Conventional versus novel antipsychotics: changing concepts and clinical implications. J Psychiatry Neurosci., 24, 431–441, 1999.

ROSSATO, J.I., KETZER, L.A., CENTURIÃO, F.B., SILVA, S.J.N., LÛDTKE, D.S., ZENI, G., BRAGA, A.L., RUBIN, M.A., ROCHA, J.B.T. Antioxidant properties of new chalcogenides against lipid peroxidation in rat brain. Neurochem Res., 27, 297–303, 2002.

ROTRUCK, J.T., POPE, A.L., GANTHER, H.E., SWANSON, A.B., HAFEMAN, D.G., HOEKSTRA, W.G. Selenium: biochemical role as a component of glutathione peroxidase. Science, 179, 588-590, 1973.

RUBINSTEIN, M., MUSCHIETTI, J.P., GERSHANIK, O., FLAWIA, M.M., STEFANO, F.J. Adaptive mechanisms of striatal D1 and D2 dopamine receptors in response to a prolonged reserpine treatment in mice. J Pharmacol Exp Ther., 252, 810-816, 1990.

SACHDEV, P., SAHAROV, T., CATHCART, S. The preventative role of antioxidants (selegiline and vitamin E) in a rat model of tardive dyskinesia. Biol Psychiatry., 46, 1672-1681, 1999.

SANTOS, F.W., ZENI, G., ROCHA, J.B.T., NASCIMENTO, P.C., MARQUES, M.S., NOGUEIRA, C.W. Efficacy of 2,3-dimercapto-1-propanesulfonic acid (DMPS) and diphenyl diselenide on cadmium induced testicular damage in mice. Food Chem Toxicol., 43, 1723-1730, 2005.

SEE, R.E. Striatal dopamine metabolism increases during long-term haloperidol administration in rats but shows tolerance in response to acute challenge with raclopride. Neurosci Lett., 129, 265–268, 1991.

SEE, R.E., CHAPMAN, M.A., KLITENICK, M.A. Chronic neuroleptic administration decreases extracellular GABA in the nucleus accumbens but not in the caudate-putamen of rats. Brain Res., 588, 177-180, 1992.

SEEMAN, P., LEE, T. Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science, 188, 1217–1219, 1975.

SEEMAN, P., VAN TOL, H.H.M. Dopamine receptor pharmacology. Curr Opin Neurol Neurosurg., 6, 602–608, 1993.

SELIM, K., KAPLOWITZ, N. Hepatotoxicity of psychotropic drugs. Hepatology, 29, 1347-1351, 1999.

SIES, H. Biochemistry of oxidative stress. Angew Chem Int Ed Engl., 25, 1058, 1986.

SIES, H. Strategies of antioxidant defense. Eur J Biochem., 215, 213, 1993.

SIES, H. Phisiological Society Symposium: Impaired endothelial and smooth muscle cell function in oxidative stress. Oxidative stress: oxidants and antioxidants. Experim Physiol., 82, 291-295, 1997.

SILVA, P. FARMACOLOGIA (7 ed.) Rio De Janeiro, Guanabara Koogan, 2006.

SMITH, R.C. Is the dopaminergic supersensitivity theory of tardive dyskinesia valid?. In: Wolf, M.E., Mosnaim, A.D. (Eds). Tardive Dyskinesia: Biological Mechanisms and Clinical Aspects. American Psychiatric Press, Washington DC, pp. 1–22, 1988.

SOARES, J.C., FOLMER, V., ROCHA, J.B.T. Influence of dietary selenium supplementation and exercise on thiol-containing enzymes in mice. Nutrition, 19, 627-632, 2003.

STEVINSON, C., ERNST, E. Valerian for insomnia: a systematic review of randomized clinical trials. Sleep Med., 1, 91-99, 2001.

TAFAZOLI, S., O'BRIEN, P.J. Peroxidases: a role in the metabolism and side effects of drugs. Drug Discov Today, 10, 617-625, 2005.

USUKI, E., PEARCE, R., PARKINSON, A., CASTAGNOL JR, N. Studies on the conversion of haloperidol and its tetrahydropyridine dehydration product to potentially neurotoxic pyridinium metabolites by human liver microsomes. Chem Res Toxicol., 9, 800-806, 1996.

VALKO, M., RHODES, C.J., MONCOL, J., IZAKOVIC, M., MAZUR, M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact., 160, 1–40, 2006.

VALKO, M., LEIBFRITZ, D., MONCOL, J., CRONIN, M.T., MAZUR, M., TELSER, J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol., 39, 44-84, 2007.

VAN KAMPEN, J.M., STOESSL, A.J. Dopamine D_{1A} receptor function in a rodent model of tardive dyskinesia. Neuroscience, 101, 629-635, 2000.

WASSER, S., LIM, G.Y., ONG, C.N., TAN, C.E. Anti-oxidant ebselen causes the resolution of experimentally induced hepatic fibrosis in rats. J Gastroenterol Hepatol., 16, 1244-1253, 2001.

WENDEL, A., FAUSEL, M., SAFAYACHI, H., TIEGS, G., OTTER., R. A novel biologically active seleno-organic compound—II Activity of PZ 51 in relation to Glutathione Peroxidase. Biochem Pharmacol., 33, 3241–3245, 1984.

WILLEY, L.B., MADY, S.P., COBAUGH, D.J., WAX, P.M. Valerian overdose: a case report. Vet Hum Toxicol., 37, 364-365, 1995.

WILSON, S.R., ZUCKER, P.A., HUANG, R.-R.C., SPECTOR, A. A development of synthetic compounds with glutathione peroxidase activity. J Am Chem Soc., 111, 5936–5939, 1989.

WOERNER, M.G., KANE, J.M., LIEBERMAN, J.A., ALVIR, J., BERGMANN, K.J., BORENSTEIN, M., SCHOOLER, N.R., MUKHERJEE, S. ROTROSEN, J., RUBINSTEIN, M., e cols. The prevalence of tardive dyskinesia. J Clin Psychopharmacol., 11, 34-42, 1991.

YANG, X., KULKARNI, A.P. Oxidation of phenothiazines by human term placental peroxidase in non-smokers. Teratog Carcinog Mutagen., 17, 139-151, 1997.

YAO, J.K., REDDY, R., MCELHINNY, L.G., VAN KAMMEN, D.P. Effects of haloperidol on antioxidant defense system enzymes in schizophrenia. J Psychiatr Res., 32, 385-391, 1998.

YASSA, R., JESTE, D.V. Gender differences in tardive dyskinesia: a critical review of the literature. Schizophr Bull., 18, 701-715, 1992.