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EFEITO DO DECOCTO DE *Bauhinia forficata* SOBRE PARÂMETROS COMPORTAMENTAIS E METABÓLICOS EM RATOS TRATADOS COM HALOPERIDOL

DISSERTAÇÃO DE MESTRADO

Luis Ricardo Peroza

Santa Maria, RS, Brasil

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EFEITO DO DECOCTO DE *Bauhinia forficata* SOBRE PARÂMETROS COMPORTAMENTAIS E METABÓLICOS EM RATOS TRATADOS COM HALOPERIDOL

Luis Ricardo Peroza

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Orientadora Profª. Roselei Fachinetto

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Elaborada por
Luis Ricardo Peroza

Como requisito parcial para obtenção do grau de
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RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

EFEITO DO DECOCTO DE *Bauhinia forficata* SOBRE PARÂMETROS COMPORTAMENTAIS E METABÓLICOS EM RATOS TRATADOS COM HALOPERIDOL

AUTOR: LUIS RICARDO PEROZA
ORIENTADORA: ROSELEI FACHINETTO

Data e Local da Defesa: Santa Maria, 27 de setembro de 2012

A esquizofrenia é uma desordem psiquiátrica que atinge cerca de 1% da população mundial e seu tratamento farmacológico consiste na utilização de antipsicóticos. Sabe-se que o tratamento crônico com antipsicóticos clássicos, como o haloperidol, pode causar distúrbios motores, dentre os quais se destaca a discinesia tardia (DT). A fisiopatologia da DT tem sido associada ao aumento do estresse oxidativo em áreas do cérebro relacionadas ao controle dos movimentos. Desta forma, estudos têm proposto o uso de compostos naturais com propriedades antioxidantes para diminuir a produção de espécies reativas na DT ou em modelos animais de discinesia orofacial (DO). A *Bauhinia forficata (B. forficata)*, uma planta utilizada na medicina popular como hipoglicemiante, e que apresenta propriedades antioxidantes, poderia ser utilizada para reduzir o estresse oxidativo presente na DO. Logo, o primeiro objetivo deste estudo foi avaliar o efeito da *B. forficata* na peroxidação lipídica *in vitro* induzida por diferentes pró-oxidantes, e também o possível efeito protetor da *B. forficata* na DO, através da quantificação dos movimentos de mascar no vazio (MMV), e na diminuição da atividade locomotora e exploratória induzidos por haloperidol em ratos (manuscrito 1). A *B. forficata* preveniu a formação de peroxidação lipídica induzida pelos agentes pró-oxidantes nitroprussiato de sódio, Fe^{2+}/EDTA e Fe^{2+}. Além disso, ratos adultos machos receberam haloperidol (38 mg/kg) a cada 28 dias por 16 semanas, e o decocto de *B. forficata* (2,5 g/L) no lugar da água de beber ou água para beber todos os dias por 16 semanas. O tratamento com o haloperidol aumentou o número de MMV, e reduziu as atividades locomotora e exploratória dos animais em relação ao grupo controle no teste do campo aberto. O co-tratamento com *B. forficata* não foi capaz de prevenir as alterações motoras induzidas por haloperidol, bem como preveniu apenas parcialmente os MMV em ratos. Por outro lado, a

B. forficata sozinha causou um aumento na atividade locomotora. Os resultados desse estudo mostraram que a *B. forficata* tem potencial antioxidante, mas esse efeito está associado apenas parcialmente à proteção contra os MMV induzidos pelo haloperidol em ratos (manuscrito 1). Alguns estudos têm mostrado que o tratamento crônico com antipsicóticos clássicos pode causar efeitos colaterais metabólicos. A *B. forficata*, conhecida na medicina popular por diminuir a glicemia, poderia prevenir os efeitos colaterais metabólicos causados pelo uso de antipsicóticos. Assim, outra proposta deste estudo foi investigar o ganho de peso, os níveis glicêmicos e outros parâmetros metabólicos em ratos tratados cronicamente com haloperidol e o possível efeito da *B. forficata* nesses efeitos colaterais metabólicos (manuscrito 2). Após 16 semanas de tratamento, os animais tratados com haloperidol apresentaram alta prevalência glicêmica e altos níveis séricos de glicose. O co-tratamento com *B. forficata* elevou os níveis de glicose e de triglicerídeos. Nenhuma diferença foi observada nos níveis de colesterol, uréia, creatinina, alanina aminotransferase, aspartato aminotransferase (AST) e lactato desidrogenase (LDH). O tratamento com haloperidol causou aumento no ganho de peso corporal e promoveu significante diminuição na taxa peso do cérebro/corpo. Em conclusão, o co-tratamento com *B. forficata* e haloperidol pode aumentar o número de animais hiperglicêmicos. Assim, é necessário enfatizar a importância de mais estudos toxicológicos sobre o tratamento crônico com *B. forficata* para evitar implicações na saúde da população.

Palavras-chave: haloperidol, discinesia tardia, discinesia orofacial, glicose, *B. forficata*, estresse oxidativo, antipsicóticos.

ABSTRACT

Dissertation of Master's Degree
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Federal University of Santa Maria, RS, Brazil

EFFECT OF *Bauhinia forficata* DECOCTION ON BEHAVIORAL AND METABOLIC PARAMETERS IN RATS TREATED WITH HALOPERIDOL

AUTHOR: LUIS RICARDO PEROZA
ADVISOR: ROSELEI FACHINETTO
Place and Date of the Defense: Santa Maria, September 27, 2012

Schizophrenia is a psychiatric disorder that affects 1% of world population and its pharmacologic treatment consists in the use of antipsychotics. It is known that chronic treatment with classical antipsychotics, such as haloperidol, can cause motors disturbers, among which stands out the tardive dyskinesia (TD). The TD pathophysiology has been associated with oxidative stress increase in areas of the brain related to the movements control. So, studies have proposed the use of natural compounds with antioxidants properties to decrease the production of reactive species on TD or on animals models of orofacial dyskinesia (OD). *Bauhinia forficata* (*B. forficata*), a plant used on folk medicine such as hypoglycemic, and presents antioxidant properties, could be used to reduce the oxidative stress present on OD. Thus, the first aim of this study was evaluate the effect of *B. forficata* on in vitro lipid peroxidation induced by pro-oxidants and also, the possible protector effect on OD, through of the quantifications of vacuous chewing movements (VCM), and on locomotor and exploratory activities decrease induced by haloperidol in rats (manuscript 1). *B. forficata* prevented the lipid peroxidation by pro-oxidant agents nitroprusside sodium, Fe^{2+}/EDTA and Fe^{2+}. Furthermore, adult male rats received haloperidol (38 mg/kg) each 28 days for 16 weeks and *B. forficata* decoction (2.5 g/L) on the place of drinking water or water to drink everyday for 16 weeks. The haloperidol treatment increased the VCM and reduced the locomotor and exploratory activities relative to control group on open field test. *B. forficata* co-treatment was not able to prevent the motor alterations induced by haloperidol, as well as only partially prevented VCM in rats. On the other hand, *B. forficata* caused an increase on locomotor activity. These results showed that *B. forficata* has antioxidant potential, but this effect is associated partially to protection against VCM induced by

haloperidol in rats (manuscript 1). Some studies have showed that chronic treatment with classic antipsychotics can cause metabolic side effects. *B. forficata* which is known on folk medicine by glycemia decrease, could prevent the metabolic side effects caused by use of antipsychotics. So, the other propound of this study was to investigate the weight gain, glycemic levels and other metabolic parameters in rats chronically treated with haloperidol and the possible effect of B. *forficata* on these metabolic side effects (manuscript 2). After 16 weeks of treatment, the animals treated with haloperidol showed high glycemic prevalence and high glucose levels. *B. forficata* co-treatment elevated glucose and triglycerides levels. No difference was observed on cholesterol, urea, creatinine, alanine aminotransferase (AST), aspartate aminotransferase (ALT) and lactate dehydrogenase (LDH). Haloperidol treatment caused weight gain and promoted a significant decrease on brain/body weight rate. In conclusion, *B. forficata* co-treatment and haloperidol can increase the number of hyperglycemic animals. Thus, is necessary to emphasize the importance of more toxicology studies on chronic treatment with *B. forficata* to avoid health implications of population.

Keywords: Schizophrenia, haloperidol, tardive dyskinesia, orofacial dyskinesia, glucose, *Bauhinia forficata*, antipsychotics.

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SUMÁRIO

APRESENTAÇÃO

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

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

O item **DISCUSSÃO** apresenta uma discussão englobando os dois manuscritos.

O iten **CONCLUSÕES ESPECÍFICAS** encontra-se no final dessa dissertação, e apresenta comentários sobre os manuscritos contidos nesse trabalho.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se às citações que aparecem nos itens **INTRODUÇÃO** e **DISCUSSÃO** dessa dissertação.

1 INTRODUÇÃO

1.1 ESQUIZOFRENIA

A esquizofrenia é uma doença mental debilitante, onde os pacientes apresentam sintomas psicóticos crônicos. Os sintomas da esquizofrenia foram divididos em sintomas positivos (desilusões, alucinações e perturbações do pensamento) e sintomas negativos (redução de interesse e de motivação e reclusão social), resultando em desorganização comportamental. Essas categorias podem ser complementadas com um grupo adicional de disfunções cognitivas (piora na atenção, aprendizagem, memória e processamento de informações) (URFER-PARNAS et al., 2010; RETHELYI, 2011).

Algumas hipóteses foram propostas para tentar explicar o desenvolvimento da esquizofrenia.

1.1.1 Hipótese dopaminérgica

A teoria da hiperdopaminergia na esquizofrenia surgiu por volta de 1960, baseada no fato de que os antipsicóticos antagonizam receptores de dopamina uma vez que seu potencial clínico está associado com sua habilidade de antagonizar receptores D2 (RD2). No entanto, a anfetamina, a qual aumenta a atividade da dopamina, pode provocar sintomas psicóticos (DAVIS et al., 1991).

Além disso, foi demonstrado que o envolvimento de outros neurotransmissores na esquizofrenia, leva em ultima instância, a alterações no sistema dopaminérgico. O papel da serotonina na esquizofrenia foi primariamente apoiado por observações que o ácido dietilamido lisérgico (LSD) causa sintomas psicóticos devido ao seu efeito serotoninérgico. Mais tarde foi esclarecido que o efeito do agonismo parcial do LSD no receptor 5-HT2A é responsável pelos sintomas psicóticos. Sabe-se também que há um aumento de receptores 5-HT2A e 5-HT1A no córtex de pacientes com esquizofrenia (AGHAJANIAN e MAREK, 2000). Não independentemente dessas evidencias, antipsicóticos atípicos, como clozapina, risperidona e olanzapina mostram alta afinidade pelos receptores 5-HT2A do que o RD2 (ARNT e SKARSFELDT, 1998), e o antagonismo de receptores 5-HT2A causa efeitos benéficos contra os sintomas negativos da esquizofrenia (SHIN et al., 2011).

Esta hipótese também incide sobre o efeito modulatório de outros neurotransmissores como o glutamato e o ácido gama-aminobutírico (GABA) no sistema dopaminérgico

(HOWES e KAPUR, 2009). O possível papel da disfunção dos receptores *N*-metil *D*-aspartato (NMDA) na esquizofrenia é apoiado por diferentes experimentos, criando a hipótese da hipofunção de NMDA (BELFORTE et al., 2011), mostrando que o antagonismo dos receptores NMDA mostra efeito similar nos sintomas positivos, negativos e cognitivos da esquizofrenia.

A relação molecular entre os receptores NMDA e o sistema dopaminérgico tem sido demonstrado em estudos *in vivo*, onde indivíduos saudáveis que receberam cetamina, um antagonista não competitivo de receptores NMDA, resultaram num aumento significante dos níveis de dopamina estriatal (NEDERGAARD et al., 1988; VOLLENWEIDER et al., 2000).

1.1.2 Modelo do neurodesenvolvimento

Fatores ambientais combinados com predisposição genética parecem ser importantes na etiologia da esquizofrenia. Traumas prematuros, como infecções pré-natais, má nutrição maternal, complicações obstétricas, em combinação com a estrutura vulnerável dos circuitos neuronais determinados por variâncias de predisposição genética, podem resultar no desenvolvimento de um cérebro fetal anormal, através de mudanças na sinapse (BALE et al., 2011; FALUDI e MIRNICS, 2011; NAGAI et al., 2011). No entanto, o desenvolvimento cerebral não termina antes do nascimento e fatores ambientais como abuso de criança, trauma de guerra, perda de pais, entre outros, podem ter similar interação com a pré-disposição genética na infância e adolescência (LARSON et al., 2011).

A esquizofrenia é uma desordem psiquiátrica marcada por uma mudança grosseira da realidade: distúrbios no pensamento, sentimentos e comportamento (DADHEECH et al., 2008; KUNZ et al., 2008; WOOD et al., 2009; PADURARIU et al., 2010). Essas condições afetam a vida social dos pacientes e por sua vez, apresentam dificuldades em se inserir na sociedade. Desta forma, os antipsicóticos consistem na alternativa farmacológica mais eficaz para o tratamento da esquizofrenia.

1.2 ANTIPSICÓTICOS

Sabe-se que antagonistas dopaminérgicos, principalmente os que bloqueiam a subclasse de RD2, promovem uma melhora nos sintomas positivos da esquizofrenia (CREESE et al., 1976). O haloperidol foi introduzido como agente terapêutico a mais de 40 anos e ainda continua sendo um dos medicamentos mais utilizado para o tratamento de psicoses agudas e crônicas. A atividade antipsicótica do haloperidol pode ser mediada, pelo

menos em parte, por sua capacidade em antagonizar o RD2 (MIYAMOTO et al., 2005). O haloperidol é também um antagonista de receptores σ1 e σ 2 (HASHIMOTO e ISHIWATA, 2006) e atua, embora com menor atividade nos receptores de dopamina D1, D3 e D4, receptor 5HT2A e receptor adrenérgicoα1 (MI YAMOTO et al., 2005). O Haloperidol exerce seus efeitos terapêuticos por agir preferencialmente no cérebro, e de fato, se acumula no tecido cerebral (KORPI et al., 1984; KORNHUBER et al., 1999). O bloqueio dos RD2 está envolvido com os efeitos colaterais motores causados pelo uso de antipsicóticos, uma vez que os antipsicóticos bloqueiam esses receptores nos gânglios basais (FARDE et al., 1992). Isso está de acordo com a hipótese que a via nigroestriatal está associado com a produção de efeitos colaterais extrapiramidais, enquanto a ação terapêutica é exercida através da via mesolímbica (ELLENBROEK, 1993). Esta falta de especificidade de regiões pode ser devido ao bloqueio de diferentes subtipos de receptores de dopamina (SEEMAN e VAN TOL, 1994).

Assim como a maioria dos antipsicóticos típicos, o haloperidol pode causar sintomas extrapiramidais, incluindo a discinesia tardia (REINKE et al., 2004).

1.3 Discinesia tardia (DT)

O termo “discinesia tardia” foi proposto pela primeira vez em 1960 por Uhrbrand e Faurbye, e é definida como uma desordem da região orofacial que resulta do tratamento crônico com antipsicóticos, e se caracteriza por movimentos involuntários repetitivos envolvendo a boca, face, e as vezes, os membros e musculatura do tronco (KANE e SMITH, 1982; WOERNER et al., 1991; YASSA e JESTE, 1992). A incidência de DT em pacientes que fazem uso de antipsicóticos varia entre 4% a 8% por ano de tratamento (GLAZER, 2000). Após 4 anos de terapia com antipsicóticos clássicos, a incidência é de 20% dos pacientes com DT, e a taxa é mais alta (até 50%) em pacientes idosos (FENTON, 2000; GLAZER, 2000; JESTE, 2000). Os mais consistentes fatores de risco para a DT são a idade, sexo feminino, o tratamento prolongado com o antipsicótico, alta dose de antipsicótico, fraca resposta ao antipsicótico, tabagismo, diabetes mellitus, sintomas extrapiramidais prematuros e história de dependência e/ou abuso de álcool (JESTE, 2000).

A DT aparece após meses ou anos subsequente ao início do tratamento com o antipsicótico, e pode persistir após a retirada da droga ou mesmo ser irreversível (CRANE, 1973; JESTE et al., 1979; CASEY, 1985b; GLAZER et al.,1990). Em animais experimentais, esta síndrome motora recebe a denominação de discinesia orofacial (DO) (ANDREASSEN e JORGENSEN, 2000). No entanto, apesar de ser bastante estudada, a etiologia da DT não é

completamente esclarecida. Existem algumas hipóteses propondo explicações para a DT e a DO.

1.3.1 Hipótese da supersensibilidade dopaminérgica

A teoria que sugere que a DT é causada pela supersensibilidade dopaminérgica pelo uso de antipsicóticos foi proposta por Carlsson (1970), Barbeu (1969 a,b), Klawans (1973), Klawans et al (1970) e Hippius e Large (1970). Segundo está hipótese, o bloqueio crônico dos receptores dopaminérgicos causaria um aumento compensatório no número de receptores, que por sua vez responderiam a níveis menores de dopamina, levando a um estado hiperdopaminérgico, o qual seria responsável pelas alterações motoras da DT. Por muitos anos está hipótese teve grande suporte, porém agora é considerada inadequada (WOLFARTH e OSSOWSKA, 1989), pois a maior limitação relacionada a está hipótese está relacionada com a idade. De fato, o envelhecimento está associado com a diminuição da função dopaminérgica e isso parece ser o principal fator de risco para a discinesia tardia (OSSOWSKA, 1993; ROTH e JOSEPH, 1994)

1.3.2 Hipótese GABAérgica

Esta hipótese está baseada em evidências de um decréscimo da atividade do ácido glutâmico descarboxilase, que catalisa a descarboxilação do glutamato para GABA, na substância negra, globus pallidus e núcleos subtalâmicos em macacos e ratos com movimentos orais induzidos por antipsicóticos (GUNNE e HAGGSTROM, 1983; GUNNE et al., 1984; JOHANSSON et al., 1990), Além disso, também foi demonstrado a diminuição no número de neurônios gabaérgicos no estriado após o tratamento crônico com antipsicóticos em ratos (PAKKENBERG et al., 1973; NIELSEN e LYON, 1978). A hipótese da GABA está suportada por estudos em ratos, onde agonistas de GABA mostraram diminuir o desenvolvimento de movimentos de mascar no vazio (MMV) induzidos por antipsicóticos (KANEDA et al.,1992; GAO et al., 1994). Há também alguns estudos em humanos onde agonistas de GABA mostraram melhorar a DT (TAMMINGA et al., 1979, TAMMINGA et al., 1983; MORSELLI et al., 1985; TAMMINGA et al.,1989), mas esta terapia não é geralmente utilizada na clínica, pois sua eficácia é baixa.

1.3.3 Hipótese da Excitotoxicidade

A hipótese da excitotoxicidade para a DT foi proposta por De Keyser, na qual o tratamento crônico com o antipsicótico poderia aumentar a liberação de glutamato para os terminais cortico-estriatais, levando à excitotoxicidade estriatal (DE KEYSER, 1991). Estudos posteriores mostraram que o tratamento crônico com antipsicóticos aumenta a liberação de glutamato no estriado e assim, também aumenta a possibilidade para a excitotoxicidade (MOGHADDAM e BUNNEY, 1993; YAMAMOTO e COOPERMAN, 1994; SEE e CHAPMAN, 1994; SEE e LYNCH, 1995). Desta forma, uma massiva ativação de receptores NMDA pelo glutamato, poderia induzir um excessivo influxo de Ca^{2+} para as células e causando efeito tóxico (NOVELLI et al., 1988).

1.3.4 Hipótese dos Radicais Livres

O estresse oxidativo (EO) tem sido proposto como um mecanismo envolvido na gênese da DT (CADET et al., 1986; CADET e KAHLER, 1994). O EO é uma condição que ocorre devido o desequilíbrio entre a produção de espécies reativas de oxigênio (ERO) e do sistema de defesa antioxidante (PADURARIU et al., 2010). Particularmente, o cérebro é vulnerável ao EO, pois possui baixos níveis de antioxidantes, altos níveis de ácidos graxos poliinsaturados e utiliza grandes quantidades de oxigênio (SULTANA e BUTTERFIELD, 2004). O EO e os produtos da peroxidação lipídica estão presentes na etiopatologia da DT (CADET et al., 1986; COYLE e PUTTFARCKEN, 1993; ANDREASSEN e JORGENSEN, 2000). É conhecido que antipsicóticos induzem um aumento do *turnover* da dopamina (SEE, 1991), o qual pode levar à formação de ERO, e que poderiam ser convertidas em radicais livres altamente reativos. O suporte para a hipótese dos radicais livres vem de estudos *in vitro* e *in vivo* aonde o haloperidol induziu EO (BEHL et al., 1996; SAGARA, 1998; POST et al., 1998). Além disso, a vitamina E atenuou as mudanças no metabolismo da dopamina no estriado induzida por antipsicóticos (JACKSON-LEWIS et al., 1991) e protegeu contra a morte celular (BEHL et al., 1995). Alguns estudos reportam que ratos que apresentam MMV induzidos por antipsicóticos, possuem altos níveis de substâncias reativas ao ácido tiobarbitúrico no estriado, sugerindo um aumento na produção de radicais livres e da peroxidação lipídica (ELKASHEF e WYATT, 1999; BALIJEPALLI et al., 2001). Todavia, não são todos os modelos experimentais que demonstram a participação direta do EO no desenvolvimento da DO (FACHINETTO et al., 2007a; 2007b) parecendo depender do tempo de tratamento para que haja ou não alteração em parâmetros oxidativos cerebrais.

Porém, mesmo sem a detecção de alterações nestes parâmetros, alguns trabalhos demonstram que o uso de antioxidantes pode minimizar o desenvolvimento da DO (BURGER et al., 2006; FACHINETTO et al., 2007b; BUSANELLO et al., 2011; BUSANELLO et al., 2012). Apesar da ampla quantidade de trabalhos a respeito da DT e DO, até o momento não há tratamento clínico efetivo para esta síndrome.

1.4 EFEITOS COLATERAIS METABÓLICOS INDUZIDOS POR ANTIPSICÓTICOS

Os efeitos colaterais metabólicos induzidos por antipsicóticos, como ganho de peso e distúrbios endócrinos, têm importantes implicações clínicas, como em aumentar significativamente a morbidade e mortalidade, reduzir a qualidade de vida, interferir com a adesão da medicação e aumentar a chance de recaída psicótica (RUSSELL e MACKELL, 2001; ALLISON et al., 2003; WEIDEN et al., 2004).

O haloperidol tem sido reportado por produzir alguns distúrbios metabólicos, incluindo ganho de peso (BOBES et al., 2003; ZIPURSKY et al., 2005), distúrbio no controle da glicose (WIRSHING et al., 2002; PEREZ-IGLESIAS et al., 2009), resistência à insulina (PEREZ-IGLESIAS et al., 2009), e piora no perfil lipídico (PEREZ-IGLESIAS et al., 2009). Também tem sido reportado por aumentar o peso corporal de ratas com ou sem aumento na ingestão de alimento (POUZET et al., 2003; FELL et al., 2004, 2005).

1.5 *Bauhinia forficata*

Durante séculos, as plantas medicinais têm sido utilizadas como remédios populares exibindo múltiplas atividades farmacológicas (JANBAZ et al., 2002; KAILEH et al., 2007). O gênero *Bauhinia* (Fabaceae), comumente conhecido como pata-de-vaca devido o aspecto bilobado das folhas, compreende mais de 500 espécies encontradas no hemisfério sul (GBIF, 2000). Diferentes espécies são usadas na medicina popular em todo o mundo no tratamento de infecções, dor e diabetes, como recentemente revisado por Cechinel-Filho (2009). A infusão de *Bauhinia forficata* tem sido utilizada como diurético, tônico, no combate à elefantíase, no controle hiperglicêmico e na redução da glicosúria (MARTINS et al., 1998). De fato essas duas últimas propriedades, relacionadas ao diabetes, tem despertado maior atenção por parte dos pesquisadores, e visando isso, muitos estudos farmacológicos foram realizados com extratos das folhas de *B. foficata* para avaliar a atividade antidiabética em diferentes modelos *in vivo* (PEPATO et al., 2002; PEPATO et al., 2004; MENEZES et al., 2007; CUNHA et al., 2010; PEPATO et al., 2010). Além da presença de flavonóides, a

presença de uma proteína *like* insulina tem sido reportada em folhas da *Bauhinia variegata*. Esta proteína reduziu a glicemia quando injetada em camundongos diabéticos (AZEVEDO et al., 2006).

Além disso, alguns estudos têm indicado que o extrato aquoso de folhas de *B. forficata* é uma potente fonte antioxidante natural e pode ser utilizado na prevenção de complicações do diabetes associado com o EO (DAMASCENO et al., 2004; KHALIL et al., 2008; VOLPATO et al., 2008). Pesquisas fitoquímicas têm revelado que esta planta contém alcalóides, taninos, mucilagem, óleo essencial, heteroglicosideos cianogenéticos, antocianinas, saponinas, catecóis e ácidos voláteis fixos (MIYAKE et al, 1986; DONATO, 1995). A *B. forficata* também apresenta atividade de *scavenger* de radicais DPPH, óxido nítrico e superóxido (FERRERES et al, 2012).

É importante enfatizar que até o momento, não há tratamento eficaz para prevenir os efeitos colaterais motores e endócrinos induzidos pelo tratamento com antipsicóticos. Deste modo, é imprescindível a realização de estudos que visem atenuar esses efeitos colaterais, principalmente com a utilização de produtos naturais com capacidade antioxidante. Desta forma, tendo em vista que as folhas de *B. forficata* são ricas em compostos fenólicos e possuem atividade hipoglicemiante, torna-se interessante estudar se o decocto de *B. forficata* poderia melhorar as alterações induzidas por antipsicóticos, a fim de que se possa posteriormente, estudar seus efeitos em pacientes que fazem uso de antipsicóticos.

2 OBJETIVOS

2.1 OBJETIVO GERAL

- Avaliar o efeito do decocto de *B. forficata* sobre alterações comportamentais e metabólicas induzidas pela administração crônica de haloperidol em ratos.

2.2 OBJETIVOS ESPECIFICOS

Experimentos *in vitro:*

- Avaliar e quantificar os componentes fitoquímicos presentes na *B. forficata.*
- Verificar a atividade antioxidante da *B. forficata* através da peroxidação lipídica induzida por pró-oxidantes.

Experimentos *in vivo* e *ex vivo* em animais tratados com haloperidol e/ou *B. forficata.*

- Avaliar o efeito da *B. forficata* nos MMV induzidos por haloperidol.
- Avaliar o efeito do tratamento na atividade locomotora.
- Investigar possíveis alterações metabólicas através de análise de parâmetros séricos.
- Analisar o ganho de peso.

3- RESULTADOS

Os resultados que fazem parte dessa dissertação estão apresentados sob a forma de dois manuscritos, os quais se encontram aqui organizados. Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas, encontram-se nos próprios manuscritos.

3.1 *Bauhinia forficata* TEM POTENCIAL ANTIOXIDANTE IN *VITRO*, MAS PREVINE PARCIALMENTE OS MOVIMENTOS DE MASCAR NO VAZIO NDUZIDOS POR HALOPERIDOL EM RATOS

Manuscrito1

Bauhinia forficata HAS ANTIOXIDANT POTENTIAL *IN VITRO*, BUT PREVENTED PARTIALLY VACUOUS CHEWING MOVEMENTS INDUCED BY HALOPERIDOL IN RATS

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ABSTRACT

Classical antipsychotics can produce motor disturbance like tardive dyskinesia in humans and orofacial dyskinesia in rodents. These motor effects have been associated to oxidative stress production in specific brain areas that occur in response to antipsychotic treatment. Thus, some studies have proposed the use of natural compounds with antioxidant properties against involuntary moviments induced by antipsychotics. In this study, we examined the possible antioxidant activity of *Bauhinia forficata*, a plant used in folk medicine as a hypoglycemic, on brain lipid peroxidation induced by different pro-oxidants. *B. forficata* prevented the formation of lipid peroxidation induced by all pro-oxidant tested. However, it was more effective against sodium nitroprusside (SNP, IC_{50} = 12.08), than Fe^{2+}/EDTA (Fe^{2+}/EDTA, IC_{50} = 41.19 μg/ml) and Fe^{2+} (Fe^{2+}, IC_{50} = 49.13 μg/ml) induced lipid peroxidation. Moreover, the effects of *B. forficata* were analyzed on an animal model of OD induced by long-term treatment with haloperidol, where adult male rats received haloperidol and each 28 days (38 mg/kg) and/or *B. forficata* decoction (2.5 g/L) for 16 weeks. Vacuous chewing movements (VCMs), locomotor and exploratory activity were evaluated. Haloperidol treatment induced VCM, and co-treatment with *B. forficata* was able to prevent this effect partially. As expected, the haloperidol administration reduced the locomotor and exploratory activity of animals in the open field test. However, *B. forficata* was not able to prevent these effects. On the other hand, *B. forficata* alone caused an increase in spontaneous locomotor activity. Our present data showed that *B. forficata* has antioxidant potential, but this effect was not associated to protection against VCM or hypolocomotion induced by haloperidol in rats. Taken together, our data suggest that the protection by natural compounds against VCM is not associated only to their antioxidant properties.

Keywords: Vacuous chewing movements; *B. forficata*; orofacial dyskinesia; locomotor activity.

1 INTRODUCTION

Haloperidol is a typical antipsychotic used in the treatment of the schizophrenia and the chronic use can cause extrapyramidal symptoms, including tardive dyskinesia (TD) in humans (Andreassen and Jorgensen 2000). It is characterized by repetitive involuntary movements involving the mouth, face, tongue and sometimes limbs and trunk musculature (Kane and Smith, 1982; Woerner et al. 1991; Yassa and Jeste, 1992). In rodents, this syndrome is named orofacial dyskinesia (OD).The molecular mechanisms related to the pathophysiology of TD or OD remain unclear. However, recent studies suggest enhanced free radicals in specific brain areas and dopamine supersensitivity collectively contribute to its pathophysiology (Seeman, 1994; Rogoza et al., 2004; Sookram et al., 2011).

Oxidative stress occurs when cellular antioxidant defense mechanisms fail to counterbalance and control endogenous reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated from normal oxidative metabolism or from pro-oxidant environmental exposures (Kohen and Nyska, 2002; Berg et al., 2004). It is described in the literature that basal ganglia, a brain area particularly involved in motor disturbances, have high concentrations of transition metals, as Cu^{2+} and Fe^{2+} (Dwork et al, 1990; Que et al, 2008). Furthermore, haloperidol is metabolized by several enzymes, including cytochrome oxidase, resulting in the generation of large quantities of oxyradicals and a potent neurotoxic pyridinium-like metabolite, further contributing to the levels of free radicals already present in the brain (Fang et al., 2001; Wright et al., 1998). Some authors have demonstrated that haloperidol-treated animals showed increased levels of thiobarbituric acid reactive substances in some brain regions, when compared to vehicle-treated control animals (Polydoro et al, 2004; Burger et al. 2005a, Fachinetto et al. 2005, Harvey et al, 2008).

Recently, attention has been given to natural products as sources of antioxidants (Newman and Cragg, 2007). It has been demonstrated that resveratrol, a phytoalexin found in grapes, cranberries, and peanuts, protects against a model of vacuous chewing movements (VCM) induced by reserpine in mice (Busanello et al, 2011) and reduced VCM induced by fluphenazine in rats(Busanello et al, 2012). Pereira et al (2011) also demonstrated the efficacy of *Valeriana officinalis* againsta model of VCM induced by reserpine in rats.

Leaves of the pantropical genus *Bauhinia* (Fabaceae) are known popularly as cow's foot, due to their unique characteristic bilobed aspect (Yeh et al., 2003). The leaves and stem-bark of these plants are used in different phytopreparations to lower blood glucose levels

(Cavalcanti and Favoreto, 2005; Mali et al. 2007). Besides, some studies have indicated that the aqueous extract of the leaves of *Bauhinia forficata* is a potential source of natural antioxidants and may be helpful in the prevention of diabetic complications associated with oxidative stress (Damasceno et al., 2004; de Sousa et al., 2004; Khalil et al., 2008). However, few is known about antioxidant properties of *B. forficata* and its action mechanisms.

Thus, the aim of this study was to evaluate the *in vitro* antioxidant potential of *B. forficata* and the use of decoction in the motor alterations induced by chronic treatment with haloperidol in rats.

2 MATERIALS AND METHODS

2.1 Animals

Male Wistar rats (±2 months old), weighing between 200 and 250 g, from our own breeding colony (Animal Householding, UFSM, Brazil) were kept in cages with free access to foods and water in a room with controlled temperature (22 ± 2°C) and in 12-h light/dark cycle with lights on at 7:00 am. This protocol was approved by internal ethical commission of UFSM under the number 025/2011.

2.2 Drugs

Tris–HCl, thiobarbituric acid and malonaldehyde bis-(dimethyl acetal) (MDA) were obtained from Sigma (St.Louis, MO, USA). Sodium nitroprusside, ferrous sulphate, ethylenediamintetracetic (EDTA), chloridric acid and acetic acid were obtained from Merck (Brazil). *B. forficata* powder was commercially obtained from Pharma Nostra (Chengdu Hawk Bio Enginnerin, China). Methanol, acetic acid, gallic acid, chlorogenic acid and caffeic acid purchased from Merck (Darmstadt, Germany). Quercetin, rutin and kaempferol were acquired from Sigma Chemical Co. (St. Louis, MO, USA).

2.3 *In vitro* experiments

2.3.1 Preparation of decoction

B. forficata powder was added to distilled water (2.5 g/L) and boiled for 10 min, before filtering it. An aliquot was used to analyses high performance liquid chromatography (HPLC) and total phenolic compounds determination.

2.3.2 Quantification of phenolic and flavonoid compounds by HPLC-DAD

HPLC-DAD was performed with the HPLC system (Shimadzu, Kyoto, Japan), Prominence Auto Sampler (SIL-20A), equipped with Shimadzu LC-20AT reciprocating pumps connected to the degasser DGU 20A5 with integrator CBM 20A, UV-VIS detector DAD (diode) SPD-M20A and Software LC solution 1.22 SP1. Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm x 250 mm) packed with 5 μm diameter particles; the mobile phase was water containing 2% acetic acid (A) and methanol (B), and the composition gradient was: 5% (B) for 2 min; 25% (B) until 10 min; 40, 50, 60, 70 and 80% (B) every 10 min. All the samples and mobile phase were filtered through 0.45 μm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.031 – 0.250 mg/ml for kaempferol, quercetin and rutin, and 0.006 – 0.250 mg/ml for gallic, chlorogenic and caffeic acids. Quantification was carried out by integration of the peaks using the external standard method, at 254 nm for gallic acid, 325 nm for caffeic and chlorogenic acids, and 365 nm for quercetin, rutin and kaempferol. The flow rate was 0.8 ml/min and the injection volume was 40 μl. The chromatography peaks were confirmed by comparing their retention time and Diode-Array-UV spectra with those of the reference standards. All chromatography operations were carried out at ambient temperature and in triplicate (Menezes et al. 2007; Silva et al. 2007).

2.3.3 Total Phenolic Compounds Determination

The total phenol content was determined by mixing the extracts with 1.25 ml 10% Folin-Ciocalteu's reagent (v/v) which was followed by the addition of 1.0 ml of 7.4% sodium carbonate. The reaction mixture was incubated at 45ºC for 15 min, and the absorbance was measured at 765 nm. Gallic acid (GA) was used as standard for phenolic compounds (Singleton et al. 1999).

2.3.4 Tissue preparation

Rats were decapitated and the cerebral (whole brain) tissue was rapidly dissected, placed on ice and weighed. Tissue was immediately homogenized in 10 mM Tris–HCl, pH

7.5 (1/10, w/v). The homogenate was centrifuged for 10 min at 4000 × g to yield a pellet that was discarded and a low-speed supernatant (S1) was used to *in vitro* analysis.

2.3.5 TBARS production

To determinate the antioxidant potential of *B. forficata*, an aliquot of 200 µl or of S1 was incubated for 1 h at 37°C and then used for lipid peroxidation determination with pro-oxidants agents in presence or absence of *B. forficata*. TBARS production was determined as described by Ohkawa et al. (1979). As pro-oxidants agents, sodium nitroprusside (SNP), Fe^{2+} and Fe^{2+}/EDTA were used.

2.4 *In vivo* experiments

2.4.1 Decoction preparation

B. forficata powder was added to tap water (2.5 g/L) and boiled for 10 min, before filtering it through a paper filter. The decoction was prepared every 2 days.

2.4.2 Treatments

Haloperidol decanoate (a slow-releasing preparation of haloperidol) or its vehicle were administered intramuscularly (i.m.) every 28 days (38 mg/kg, i.m.), which is equivalent to 1 mg/kg/day of unconjugated haloperidol (Fachinetto et al, 2005; Fachinetto et al, 2007b). *B. forficata* decoction was given in place of drinking water at a final concentration of 2.5 g/L, in a dose equivalent to 250-300 mg/Kg/day (equivalent to 2.04-2.45 mg of gallic acid/kg/day). A low dose was used to avoid side effects. The total time of treatment was 16 weeks.

The rats (n=30) were divided into four groups: control group (n=5) received soy oil (i.m.), which was the haloperidol vehicle, and drinking water; *B. forficata* group (n=5) received soy oil (i.m.) and *B. forficata* decoction; haloperidol group (n=10) received haloperidol decanoate (i.m.) and drinking water; and haloperidol plus *B. forficata* group (n=9) received haloperidol decanoate (i.m.) and *B. forficata* decoction. Consumption of *B. forficata* decoction was observed throughout the treatment to allow calculate the dose.

2.4.3 Behavioral analysis

2.4.3.1 Open field test

To analyze changes in spontaneous locomotor activity caused by treatment with haloperidol and/or *B. forficata*, the animals were placed individually in the center of an open field arena (40×40×30 cm) with black plywood walls and a white floor divided into 9 equal squares, as previously described (Broadhurst, 1960). The number of line crossings and the number of rears was measured over 5 min and taken as an indicator of locomotor activity.

2.4.3.2 Quantification of VCMs

Behavior measurement of VCMs was assessed before the treatment with haloperidol or its vehicle (basal evaluation), as previously described (Fachinetto et al., 2007b). The effect of the drugs on behavior was examined after 16 weeks at the end of the experimental period. To quantify the occurrence of VCMs, rats were individually placed in cages (20×20×19 cm) to quantify VCMs frequency. VCMs are defined as single mouth openings in the vertical plane not directed towards physical material. If VCMs occurred during a period of grooming, they were not taken into account. The behavioral parameters of OD were measured continuously for 6 min after a period of 6 min adaptation. During the observation sessions, mirrors were placed under the floor of the experimental cage to allow observation when the animal was faced away from the observer. Experimenters were always blind to treatments.

2.5 Statistical Analisys

Data were analyzed by one-way or two-way ANOVA, followed by Post Hoc test when appropriate. Significance was considered when $p < 0.05$.

3 RESULTS

3.1 HPLC analysis

HPLC fingerprinting of the *B. forficata* decoction showed an elution diagram when the peaks were grouped into three regions based on the UV absorption profile. These regions showed typical patterns of UV absorption, supporting the presence of gallic acid (8.26%, peak 1), chlorogenic acid (2.73%, peak 2), caffeic acid (5.92%; peak 3), rutin (1.14%; peak 4), isoquercitrin (8.15 %; peak 5), quercetin (5.89%; peak 6) and kaempferol (2.27%; peak 7), (Fig. 1 and Table 1). Therefore, HPLC analysis revealed that hydrolysable tannins, flavonoids and phenolics are the major components of the extract. The results are in agreement to other studies (Menezes, et al. 2007; Silva et al. 2007).

3.2 Effects of *B. forficata* decoction on brain lipid peroxidation induced by iron

Statistical analyzes revealed that Fe^{2+} induced a significant increase in brain homogenate TBARS levels ($p < 0.0001$), which were reduced by *B. forficata* decoction in a concentration dependent manner ($p < 0.05$; Fig. 2).

When lipid peroxidation was induced by Fe^{2+}/EDTA, it was also observed a significant increase in TBARS levels ($P < 0.0001$; Fig. 3) that were reduced by *B. forficata* decoction ($P < 0.05$) in a concentration dependent manner.

With SNP as a pro-oxidant agent, it was observed a significant increase on TBARS formation in brain homogenates ($p<0.0001$) that was reduced in a concentration dependent manner to basal levels by *B. forficata* decoction ($p < 0.05$; Fig.4). However, it was possible to verify that the *B. forficata* reduced the TBARS formation to basal levels with a minor concentration than when TBARS was induced by Fe^{2+} or Fe^{2+}/EDTA being confirmed by an IC_{50} of 12.08 µg/mL (Table 2). The IC_{50} of *B. forficata* to inhibit TBARS formation was Fe^{2+}>Fe^{2+}/EDTA>SNP as shown in table 2.

3.3 Effects of *B. forficata* on VCMs induced by long-term treatment with haloperidol

Chronic treatment with haloperidol increased the number of VCM compared to its vehicle ($p<0.05$; figure 5). Co-treatment with *B. forficata* was able to reduce partially VCMs in rats.

3.4 Effects of long-term treatment with *B. forficata* and haloperidol on locomotor activity in rats

Haloperidol decreased markedly the number of crossing ($p < 0.05$; Fig. 6A) in the open field test after 16 weeks of treatment that was not reversed by co-treatment with *B. forficata*. Surprisingly, *B. forficata* alone increased significantly the number of crossings ($p < 0.05$; Fig. 6A). When the number of rearing in the open field test was monitored, haloperidol caused a decrease in the number of rearing ($p < 0.05$; Fig. 6B) in the open field test after 16 weeks of treatment. However, in these parameters, *B. forficata* did not cause a significant effect on the number of rears neither alone nor co-administrated with haloperidol.

4 DISCUSSION

Natural products have been target of several studies, mainly due to their pharmacological properties, low toxicity and high acceptance by the population. The first aim of the present study was investigate the effects of *B. forficata*, a plant which is popularly used to decrease glycemia and that presents antioxidant compounds, on brain lipid peroxidation induced by pro-oxidants. *B. forficata* prevented the formation of lipid peroxidation induced by all pro-oxidants.

It is known that the brain is vulnerable to oxidative damage because of a relative lack of antioxidant enzymes like catalase and glutathione peroxidase and abundance of oxidizable substrates like polyunsaturated fatty acids, catecholamines etc. (Halliwell and Gutteridge, 1985; Cohen, 1988).

Of note, the results presented here show that decoction of *B. forficata* prevents oxidative damage in brain homogenates induced by different pro-oxidants SNP, Fe^{2+} and Fe^{2+}/ EDTA complex, indicating that the *B. forficata* decoction has high antioxidant activity. When SNP was used to induce lipid peroxidation, *B. forficata* concentration (25 μg/ml) required to reduce TBARS to basal levels was lower when compared with concentrations required in the presence of free iron or Fe^{2+}/EDTA (250 μg/ml). Also, the inhibitory concentration of *B. forficata* able to inhibit the lipid peroxidation induced by pro-oxidants was calculated and showed be lower to nitroprusside sodium (SNP, IC_{50} = 12.08), than Fe^{2+}/EDTA (Fe^{2+}/EDTA, IC_{50} = 41.19 μg/ml) and Fe^{2+} (Fe^{2+}, IC_{50} = 49.13 μg/ml). These results can be due the presence of high concentration of quercetin found on *B. forficata* decoction. It is consistent with Wagner et al (2008), which quercitrin reduced lipid peroxidation *in vitro*.

SNP has been suggested to cause citotoxicity via the release of cyanide and/or nitric oxide (Bates et al., 1991; Chen et al., 1991; Dawson et al., 1991; Rauhala et al., 1998). After the release of NO, the iron moiety may react with SNP, which could lead to the formation of highly reactive oxygen species, such as ˙OH via the Fenton reaction (Graf et al., 1984), which may cause oxidative stress and nigrostriatal injury in the rat brain (Rauhala et al. 1998). The iron and the complexes Fe^{3+}/EDTA have similar pro-oxidant properties to SNP, which can generate ˙OH radicals and initiate lipid peroxidation (Halliwell and Gutteridge, 1989; Dean et al., 1997; Rauhala et al., 1998). Brain is extremely sensitive to iron overload and intracerebral injection of Fe^{2+} causes neurotoxic effects (Bostanci and Bagirici, 2008).

Haloperidol blocks dopamine D2 autoreceptors as well as postsynaptic receptors in the striatum and other brain regions, which is followed by a compensation effect of increased dopamine synthesis and release in the striatum (Lerner et al., 1977). Elevated levels of dopamine and its metabolites can be oxidized, via the Fenton reaction to generate increased concentrations of ROS (Rogoza et al., 2004) or metabolized by monoamine oxidase (MAO) action, with production of H_2O_2 (Mariani, et al, 2005). It has been demonstrated that oxidative stress could be involved in these abnormal movements (Abílio et al. 2004; Andreassen et al. 2003; Burger et al. 2005a,b; Faria et al. 2005; Naidu et al. 2003). In another study, it was demonstrated that *Ilex paraguariensis* was able to reduce both oral movements and oxidative stress induced by haloperidol in rats after acute treatment (Colpo et al. 2007).

Thus, the second aim of this study was investigate the effects of *B. forficata* on VCM and on alterations of locomotor and exploratory activity induced by chronic haloperidol treatment in rats. We found that long-term treatment with haloperidol caused a prevalence of OD in 50% of the treated rats (data not shown). This data is in accordance with our previous work (Fachinetto et al. 2007b). However, no significant effect of *B. forficata* on reducing prevalence (data not shown) but only intensity of VCM induced by haloperidol in rats was partially prevented. This data suggests that the antioxidant activity of *B. forficata* is not necessarily the main reason for its efficacy in VCMs model.

The effects of haloperidol and/or *B. forficata* on spontaneous locomotor activity in the rats in an open field test were also investigated. This test is commonly used to confirm the efficacy of dopamine antagonist drugs. As expected, haloperidol decreased locomotor parameters, crossing and rearing. However, to our surprise, *B. forficata* caused a significant increase in crossings but not in rearings. To our knowledge, this data is reported for the first time in literature. It is probable that a constituent of this plant is acting in specific regions of the brain. It is known that suppression of locomotion by haloperidol results from dopaminergic pathway blockage in nucleus accumbens (Kelley et al. 1989; Salamone et al. 1998). However, the mechanisms involved in the effect of *B. forficata* on crossings need to be further investigated.

CONCLUSION

B. forficata decoction decreased pro-oxidants- induced lipid peroxidation. However, *B. forficata* partially reducing haloperidol-induced VCM and not even improved the hypolocomotion caused by haloperidol treatment. The long-term treatment with *B. forficata* alone caused hyperlocomotion in the rats. It is important to highlight the need to study natural

products, since their indiscriminate use by the population may produce health risks. Moreover, further studies are necessary to investigate the mechanism of action of this plant.

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LEGENDS FOR FIGURES AND TABLES:

Figure 1 - Profile of *B. forficata* by HPLC analysis. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rutin (peak 4), isoquercitrin (peak 5), quercetin (peak 6) and kaempferol (peak 7). Calibration curve for gallic acid: Y = 53985x + 1020.6 (r = 0.9859); chlorogenic acid: Y = 52548x + 1082.3 (r = 0.9850); caffeic acid: Y = 87846x + 1093 (r = 0.9938); rutin: Y = 103861x – 1235.8 (r = 0.9921); quercetin: Y = 150833x – 4741.7 (r =0.9949) and kaempferol: Y = 130745x – 1897.9 (r = 0.9928).

Figure 2 - Effects of *B. forficata* (0-250 µg/ml) decoction on Fe^{2+} (100 µM)-induced TBARS production in rat brain homogenates. Values are expressed as mean ± SEM of 3 independent experiments performed in duplicate. Data were analyzed by one-way ANOVA followed by Tukey test. $^{\#}$ different from basal; *,**,*** different from induced by Fe^{2+}.

Figure 3 - Effects of *B. forficata* decoction (0-250 µg/ml) on Fe^{2+}/EDTA(100 µM)-induced TBARS production in rat brain homogenates. Values are expressed as mean ± SEM of 3 independent experiments performed in duplicate. Data were analyzed by one-way ANOVA followed by Tukey test. $^{\#}$ different from basal; *,**,***,**** different from induced by Fe^{2+}/EDTA.

Figure 4 - Effects of *B. forficata* decoction (0-25 µg/mL) on SNP (5 µM)-induced TBARS production in rat brain homogenates. Values are expressed as mean ± SEM of 3 independent experiments performed in duplicate. Data were analyzed by one-way ANOVA followed by Tukey test. $^{\#}$ different from basal; *,**,*** different from induced by SNP.

Figure 5 - Effects of *B. forficata* on haloperidol-induced orofacial dyskinesia in rats after 16 weeks of treatment (number of VCM during 6 min). Values are presented as means±S.E.M. (Control, n=5; *B. forficata*, n=5; haloperidol, n=10; haloperidol+ *B. forficata*, n=10. *different from control and *B. forficata* groups; $^{\#}$ different from haloperidol and haloperidol + *B. forficata* groups.

Figure 6 - Effects of *B. forficata* and/or haloperidol on open field test in rats. Number of crossings in 5 min. Values of number of crossings are presented as means±S.E.M. (Control, n=5; *B. forficata*, n=5; haloperidol, n=10; haloperidol+ *B. forficata*, n=10). One-way ANOVA followed by Tukey test. * represents significant differences from control group; $^{\#}$ represents significant differences from control and *B. forficata* groups.

Table 1: Phenolics and flavonoids composition of *B. forficata*. Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$.
* Quantified as quercetin

Table 2 – IC50 values of *B. forficata* against different pro-oxidant agents. Values are expressed as mean ± SEM.

FIGURE 1:

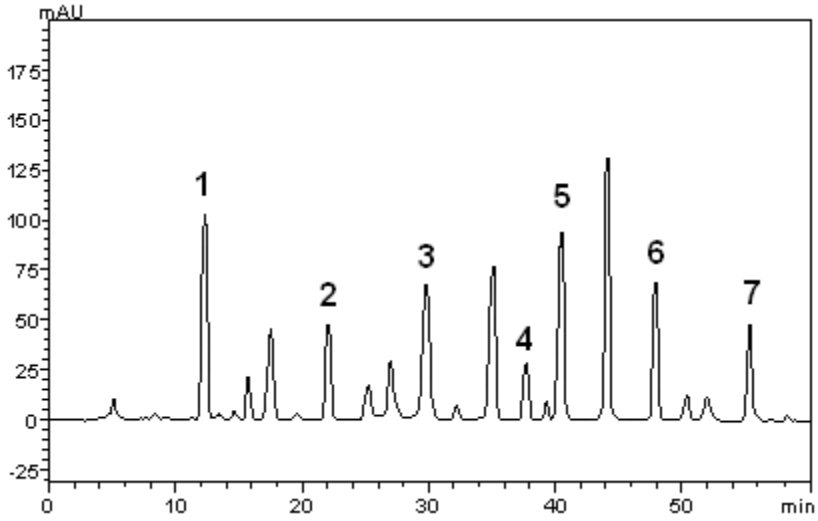
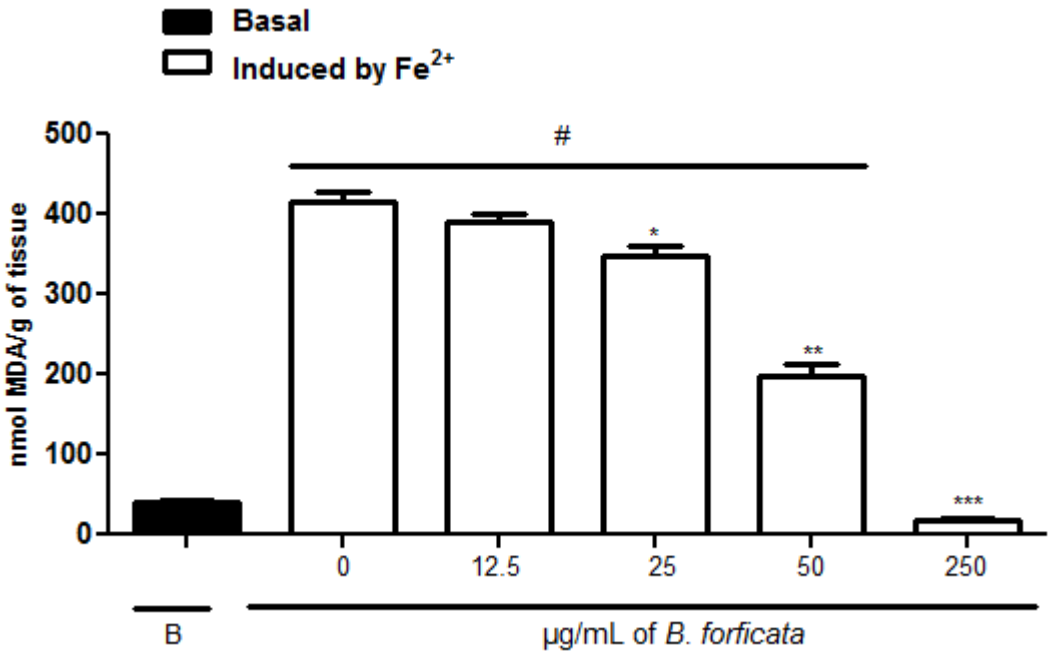


FIGURE 2:


Basal
Induced by Fe2+
nmol MDA/g of tissue
500
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200
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0
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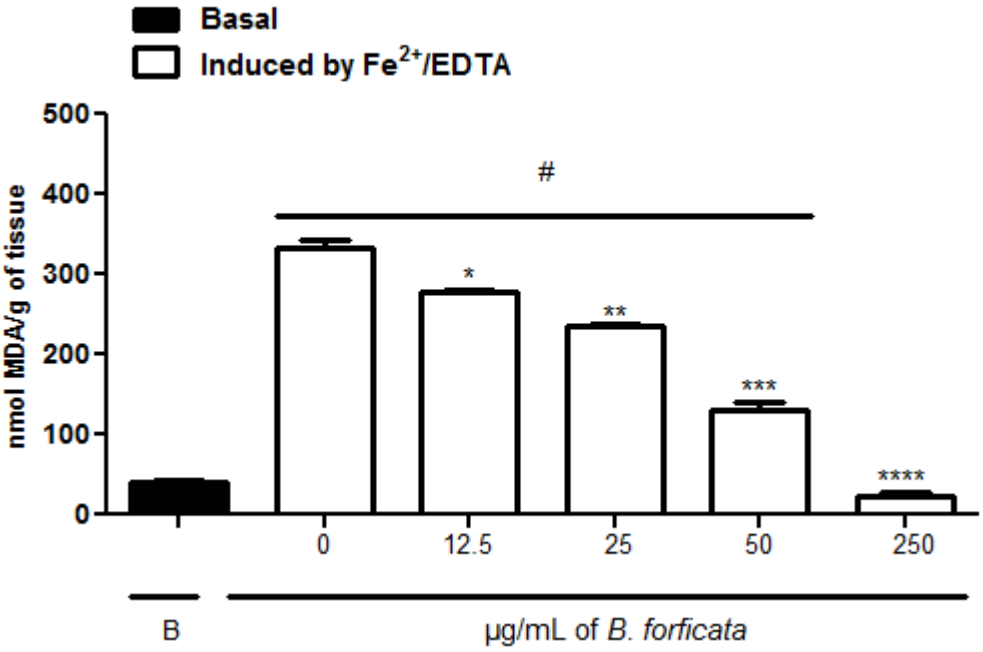
0
12.5
25
50
250
B
µg/mL of B. forficata

FIGURE 3:


Basal
Induced by Fe2+/EDTA
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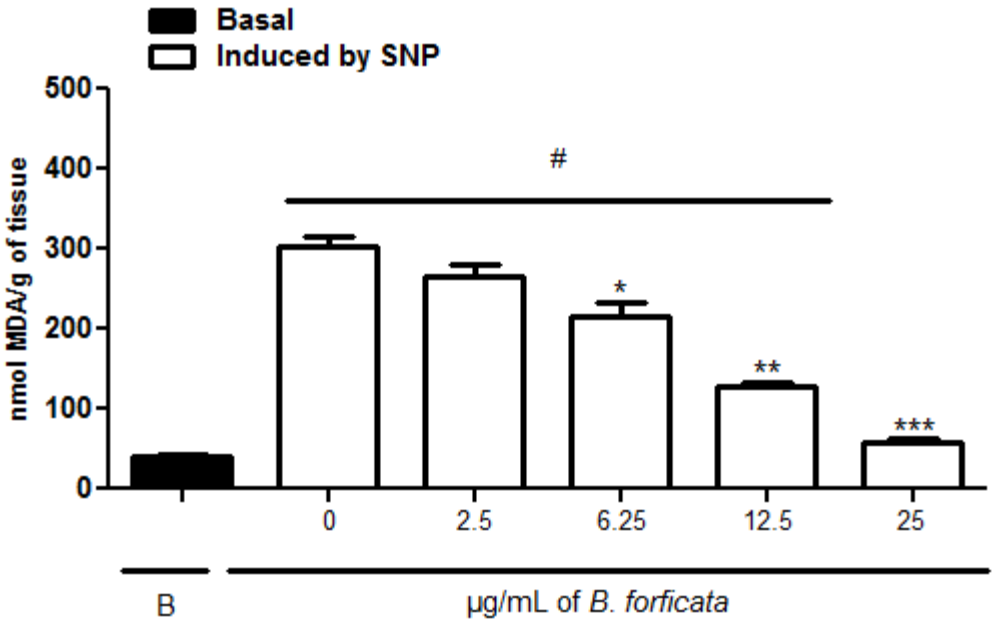
µg/mL of B. forficata

FIGURE 4:


Basal
Induced by SNP
nmol MDA/g of tissue
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400
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0
2.5
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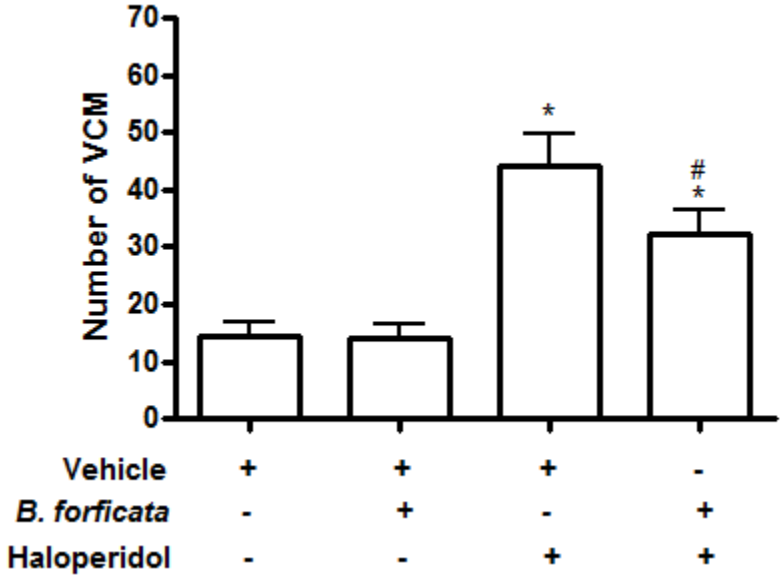
µg/mL of B. forficata

FIGURE 5:


Number of VCM
70
60
50
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*
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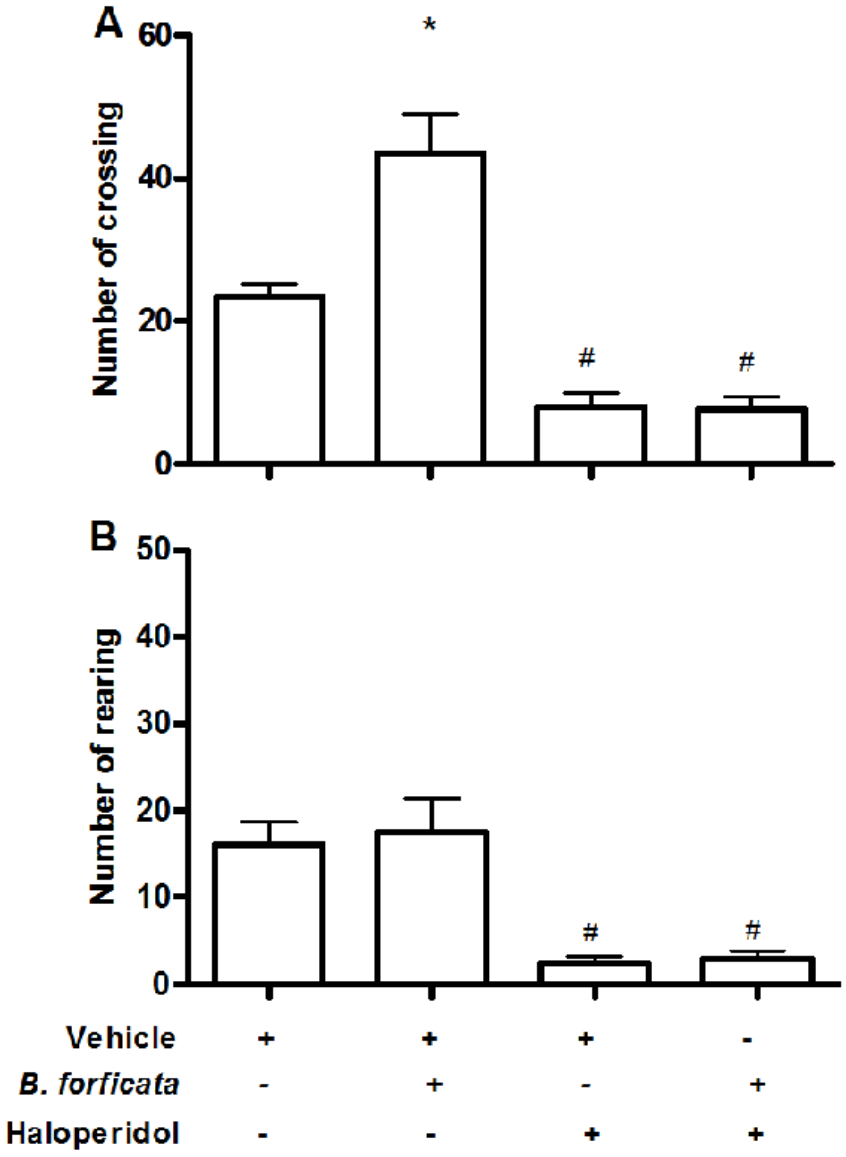
Vehicle + + + -
B. forficata - + - +
Haloperidol - - + +

FIGURE 6: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Vehicle + + + -
B. forficata - + - +
Haloperidol - - + +

TABLE 1:

Compounds	*B. forficata*	
	mg/g	Percent
Gallic acid	82.6 ± 0.12 a	8.26
Chlorogenic acid	27.3 ± 0.02 b	2.73
Caffeic acid	59.2 ± 0.09 c	5.92
Rutin	11.4 ± 0.26 d	1.14
Isoquercitrin*	81.5 ± 0.34 a	8.15
Quercetin	58.9 ± 0.15 c	5.89
Kaempferol	22.7 ± 0.08 e	2.27

TABLE 2

Pro-oxidant	**IC_{50}**
Fe^{2+} (100 µM**)**	49.13±1.726 µg/mL
Fe^{2+}/EDTA (100 µM)	41.19±0.728 µg/mL
SNP (5 µM)	12.08±2.083 µg/mL

3.2 *Bauhinia forficata* AUMENTA *A PREVALÊNCIA DE HIPERGLICEMIA EM RATOS SOB TRATAMENTO CRÔNICO COM HALOPERIDOL*

Manuscrito 2

Bauhinia forficata INCREASES THE PREVALENCE OF HYPERGLYCEMIA IN RATS UNDER CHRONIC HALOPERIDOL TREATMENT

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ABSTRACT

Classic antipsychotics are used on schizophrenia treatment and are effective against positive symptoms of this psychiatric disorder. However, antipsychotics can produce metabolic side effects. *Bauhinia forficata* is a plant used in folk medicine as hypoglycemic and has been used in studies to reduce blood glucose in rats. Thus, the purpose of this study was to investigate the glycemic levels and other metabolic parameters in rats receiving long-term treatment with haloperidol, a classic antipsychotic, and/or *B. forficata*. Adult male rats were randomly divided into 4 groups, such as control, *B. forficata* (250-300 mg/kg/day, p.o.), haloperidol (38 mg/Kg, i.m.), and haloperidol + *B. forficata* groups. Body weight gain was measured throughout of the treatment. After 16 weeks of treatment, the fasting blood glucose, cholesterol, triglycerides, urea, creatinine, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase levels were quantified in rats. Haloperidol treatment increased body weight gain and promoted a significant decrease in brain/body weight ratio. *B. forficata* co-treatment induced a higher prevalence of hyperglycemia and high levels of triglycerides in rats. No statistical differences were observed in levels of other analyzed biochemical parameters of animals chronically treated with haloperidol and/or *B. forficata*. In conclusion, *B. forficata* co-treatment with haloperidol may produce hyperglycemia in rats. Thus, it is necessary to emphasize the importance of addicional toxicological studies concerning chronic treatment *B. forficata* to avoid population health implications.

Keywords: Glucose; *B. forficata*; metabolic side effects; haloperidol; triglycerides.

Introduction

Schizophrenia is a prevalent psychiatric illness in the population around the world (Kirkbride et al, 2012). The treatment consists in the utilization of typical and atypical antipsychotic (Snyder et al, 1975). However, the clinical efficacy is frequently compromising due to innumerable side effects, as metabolic disruptions, evolving the chronic treatment of the patients with these drugs (Gold et al, 1991; Alisson et al, 1999; Andreassen and Jorgensen, 2000)

These side effects have important clinical implications because they significantly increase morbidity and mortality, reduce quality of life, interfere with medication compliance and increase the chance of psychotic relapse (Alisson et al, 1999; Russell and Mackell, 2001; Allison et al, 2003). Besides, metabolic alterations in patients to be commonly associated to use of atypical antipsychotics (Russel and Mackell, 2001), treatment with typical antipsychotics may also alter metabolism (Hagg et al, 2001; Savoy et al, 2010). There are many studies comparing if the development of metabolic side effects in humans appears more frequently in those patients taking typical or taking atypical antipsychotic (Buse et al, 2003; Feldman et al, 2004; Miller et al, 2005; Ostbye et al, 2005 . However, there is not a consensus about this because it was not possible to verify statistical differences between metabolic side effects developed by typical or atypical antipsychotics in humans. The fact is that both classes of these antipsychotics are associated with metabolic changes, such as body weight gain and endocrine disruptions (Henderson, 2002).

In this context, haloperidol, a typical antipsychotic, is a butyrophenone that acts primarily as a D2 dopamine receptor antagonist (Creese et al, 1975). It has been shown that high concentrations of this dopamine D2 receptor antagonist are cytotoxic for various cell types (Vilner et al, 1995). Some literature data have shown that, in humans and rodents, haloperidol treatment interferes with the metabolism through an imbalance in insulin and glucagon levels (Malhotra et al, 1993; Lin et al, 2006), interfering, consequently, with the metabolism of carbohydrates, lipids and proteins. However, there are discrepancies among the studies and differences in the duration of treatments.

Medicinal plants exhibit multiple pharmacological activities and have been studied as therapeutic adjuvant. The genus *Bauhinia* (Fabaceae) comprises more than 500 species widespread found in the southern hemisphere (GBIF, 2000), their leaves are known popularly as cow's foot, due to their unique characteristic bilobed aspect. Studies have demonstrated the efficacy of *Bauhinia forficata (B. forficata)* in reduce glycemia in different models of acute

and subchronic hyperglycemia (Panda and Kar, 1999; Lino et al, 2004). The hypoglycemic mechanism proposed to *B. forficata* is an increase of sensitivity of cells to any plasmatic residual insulin present in the rats (Pepato et al, 2002; Jorge et al, 2004). However, there are neither data about effects of chronic treatment with *B. forficata* on glycemia in rats nor about the possible association of *B. forficata* as adjuvant treatment with antipsychotic drugs with the objective to minimize metabolic side effects.

It is important to emphasize that at present, there are no data about prevalence of the hyperglycemia in rats receiving chronic treatment with haloperidol. Thus, the purpose of this study was to investigate the glycemic levels and other metabolic parameters in rats receiving long-term treatment with haloperidol and/or *B. forficata* and the possible protective effect of *B. forficata* on metabolic side effects caused by chronic treatment with haloperidol.

Material and Methods

Animals

Male Wistar rats (±2 months old) weighing between 270 g and 320 g, from our own breeding colony were housed in a room with controlled temperature (22±3 °C) and 12:12-h light/dark, with continuous access to food and drinking water or *B. forficata* decoction. They were maintained and used in accordance to the guidelines of the Brazilian Association for Laboratory Animal Science following the law 11.794/08. This protocol was approved by internal ethical commission of UFSM under the number 025/2011.

Drugs

Haloperidol decanoate was commercially acquired (Haldol®, Cristália, São Paulo, Brazil). *B. forficata* powder was obtained commercially from Pharma Nostra (Chengdu Hawk Bio Enginnerin, China). Methanol, acetic acid, purchased from Merck (Darmstadt, Germany).

Treatments

Haloperidol decanoate (a slow-releasing preparation of haloperidol) or its vehicle were administered intramuscularly (i.m.) every 28 days (38 mg/Kg, i.m.), which is equivalent to 1 mg/kg/day of unconjugated haloperidol (Fachinetto et al, 2005; Fachinetto et al, 2007). *B. forficata* decoction was given in place of the drinking water at a final concentration of 2.5 g/L, in a dose equivalent to 250-300 mg/Kg/day (equivalent to 2.04-2.45 mg of gallic acid/Kg/day). A low dose was used to avoid side effects.

The rats were divided into four groups: control group (n=4) received soy oil (i.m.), which was the haloperidol vehicle, and drinking water; *B. forficata* group (n=5) received soy oil (i.m.) and *B. forficata* decoction; haloperidol group (n=10) received haloperidol decanoate (i.m.) and drinking water; and haloperidol plus *B. forficata* group (n=9) received haloperidol decanoate (i.m.) and *B. forficata* decoction. Consumption of *B. forficata* decoction was observed throughout the treatment to allow calculating the dose. Weight gain was also monitored during the whole experimental period.

Decoction preparation

B. forficata powder was added to water (2.5 g/l) and boiled for 10 min, allowing the decoction to stand for 30 min before filtering it through a paper filter. The decoction was prepared every 2 days. An aliquot was used to qualitative analyses by HPLC and total phenolic compounds determination (data not shown).

Tissue preparations

Rats were killed on the 28th day after the last administration of haloperidol. The brain, liver and kidneys were immediately excised, put on ice and weighed to calculate the ratio organ weight/body weight.

Biochemicals parameters

After 16 weeks of treatment, animals were fasted overnight and killed by decapitation. Free running blood samples (6 ml) were collected. Serum levels of glucose, triglyceride, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine and urea were determined with commercial assay Bioclin®. We observed that some rats presented glycemic levels higher than 200 mg/dL, which characterizes hyperglycemia. Therefore, in order to represent the obtained results in a proper way, we separated the animals into groups with >200 mg/dL and <200 mg/dL and calculated the prevalence (Fig. 3).

Statistical analysis

Data from biochemical parameters were analyzed by two-way ANOVA, followed by Duncan's Post Hoc test when appropriate. Significance was considered when $p < 0.05$.

Results

Effects of *B. forficata* and/or haloperidol on bodyweight gain in rats

B. forficata treatment had no effect on body weight gain (Fig. 1). However, haloperidol treatment increased body weight gain in the rats, which was evidenced by two-way ANOVA (time X treatment; $F(5, 21) = 3.44$ and $p < 0.05$).

Effect of *B. forficata* and/or haloperidol on brain, liver and kidney weight

Two-way ANOVA of brain/body weight ratio demonstrated a significant main effect of haloperidol treatment ($F(1,27) = 5.30$ and $p < 0.01$), since the haloperidol treatment caused a significant decrease in brain/body weight ratio. Duncan's multiple range test also indicated that haloperidol-treated rats had a significantly lower brain/body weight ratio than *B. forficata* group (Table 1). No statistical effect was seen in brain weight.

As in brain weight, any other statistical significance was not seen neither in liver and kidney weights nor in liver/body and kidney/body weight ratio.

Effects of long-term treatment with *B. forficata* and haloperidol on plasmatic biochemical parameters in rats

No significant effect on glucose levels was observed when the animals were divided in four groups (Fig. 2). However, high discrepancies in glycemic levels were seen within the same experimental group. Hence, we analyzed the animals presenting glycemia < 200 mg/dL separately from those presenting glycemia > 200 mg/dL. It could be observed that haloperidol treatment caused a low prevalence of hyperglycemia with about 20% of the animals presenting levels higher than 200 mg/dL (Fig. 3). Unexpectedly, chronic treatment with *B. forficata* alone caused a prevalence of 40% of the animals presenting glucose levels higher than 200 mg/dL and haloperidol co-treatment with *B. forficata* caused a prevalence of about 55% of the animals presenting high plasmatic glucose levels (Fig. 3).

Two-way ANOVA revealed a significant main effect of haloperidol on triglycerides ($F(1,25) = 3.91$ and $p = 0.05$) levels. Duncan's multiple range test evidenced a significant increase in triglycerides levels of haloperidol+ *B. forficata* treated animals compared to control groups (Table 2).

No statistical differences were observed in creatinine, cholesterol, ALT, AST, urea and LDH levels of animals chronically treated with haloperidol and/or *B. forficata*.

Discussion

Natural products have been target of several studies, mainly due to their pharmacological properties, low toxicity and high acceptance by the population. In this study, we evaluated the possible beneficial effect of *B. forficata* on possible metabolic effects induced by haloperidol.

The present study investigated the glycemic levels and other metabolic parameters in rats receiving long-term treatment with haloperidol and/or *B. forficata* and the possible protective effect of *B. forficata*, a plant which is popularly used to decrease glycemia and that presents antioxidant properties. We also reported, for the first time in literature, that long-term *B. forficata* co-treatment with haloperidol induced a higher prevalence of hyperglycemia than haloperidol alone. Furthermore, we showed the prevalence of hyperglycemia in rats taking long-term treatment with haloperidol is about 20%.

There are several studies showing that neuroleptic drugs may cause metabolic damage (Hagg et al, 2001; Henderson, 2002; Savoy et al, 2010). It has been demonstrated that haloperidol can accumulate in brain tissue (Korpi et al, 1984; Kornhuber et al 1999) and produce metabolic disturbances, including weight gain (Bobes et al, 2003; Zipursky et al, 2005), impaired glycemic control (Wirshing et al, 2002; Perez-Iglesias et al, 2009), insulin resistance and a worsening of the lipid profile (Perez-Iglesias et al, 2009). Nucleus accumbens D1/D2 receptor blockade affected motor behavior, had small effects on feeding patterns, but did not reduce the amount of food consumed (Baldo et al, 2002). Some studies have shown the involvement of D2 receptor on metabolic parameters. Garcia-Tornadu et al (2010) studied the glucose homeostasis in D2 receptor knockout mice (*Drd2*-/-). Mice *Drd2*-/- presented decrease insulin response to glucose, high fasting glucose and were glucose intolerant.

Of particular interest, haloperidol might affect glucose homeostasis through several distinct mechanisms. Firstly, haloperidol, as well as other antipsychotics, dramatically reduces physical activity (Simon et al, 2000; Wiley and Evans, 2008; Albaugh et al, 2011), and impaired physical activity might directly diminish insulin sensitivity. Secondly, haloperidol consistently elevates serum concentrations of prolactin (Hruska, 1986; Xu et al, 2002), and this is associated with glucose intolerance and insulin resistance (Gustafson et al, 1980; Tuzcu et al, 2009). However, there are no data in the literature about the percentage of rats treated with haloperidol and effectively showing metabolic disturbances. Therefore, we investigated body weight gain and plasmatic biochemical parameters to determine whether haloperidol

could alter mainly metabolism of carbohydrate and lipids in our experimental protocol. In fact, haloperidol treatment increased body weight gain in the rats and promoted a significant decrease in brain/body weight ratio.

When the glycemia of the treated animals was analyzed, we had another unexpected effect. Haloperidol treatment caused a low prevalence of hyperglycemia with about 20% of the animals presenting levels higher than 200 mg/dL. However, for the first time, we demonstrated that chronic treatment with *B. forficata* alone caused a prevalence of 40% of the animals presenting levels of glucose higher than 200 mg/dL. In addition, haloperidol co-treatment with *B. forficata* caused a prevalence of about 55% of the animals presenting high plasmatic glucose levels. It is important to emphasize that *B. forficata* constituents or metabolites apparently interact with haloperidol, and such interaction is harmful to the animals. Furthermore, *B. forficata* alone seems to decrease the sensitivity of the organism to glucose, triggering hyperglycemia in rats under long-term treatment. Literature data suggests that *B. forficata* acts as an insulin mimetic agent, increasing the release of insulin from the pancreatic β-cells (Frankish et al, 2010).

An *in vitro* study showed that kaempferitrin (kaempferol 3,7-dirhamnoside), a flavonoid extracted from *Bauhinia acuminata*, inhibits the translocation of Glucose Transporter 4 (GLUT4) stimulated by insulin, and also glucose uptake by inactivating protein kinase B/Akt that is involved in signaling translocation of GLUT4, and by locking directly in glucose transport channel on GLUT4 in 3T3-L1 adipocytes (Prasad et al, 2009). Other studies show that haloperidol negatively modulates Akt signaling mediated by insulin in cultured neurons and PC12 cells (Ukai et al, 2004; Dai et al, 2007). Thus, other hypothesis for the hyperglycemic found in this study could be caused due to the presence of kaempferol found in *B. forfcata,* which would prevent the translocation of GLUT4 in adipose and muscle tissue, leading to hyperglycemia. An even worse hyperglycemia was observed in rats haloperidol treated and *B. forficata* co-treated, because once haloperidol negatively modulates expression of Akt in insulin signaling, this effect could be enhanced by the presence of kaempferol in *B. forficata*.

Since an increase in glycemia levels of the animals treated with *B. forficata* was observed in this study, we can suppose that long-term treatment may induce resistance of the cells in utilizing glucose. Furthermore, we also verified an increase in triglycerides levels when animals were co-treated with haloperidol and *B. forficata* suggesting another toxic effect on metabolism.

In conclusion, our data suggest that long-term haloperidol treatment could induce moderate alterations in the metabolism and *B. forficata* co-treatment was able to exacerbate these effects. It is important to highlight the need to study natural products, since their indiscriminate use by the population may produce health risks. Moreover, further studies are necessary to investigate the mechanism of action of this plant.

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Legends for figures and tables

Figure 1: Bodyweight gain in rats under treatment with *B. forficata* and/or haloperidol during 16 weeks.

Figure 2: Effects of long-term treatment of *B. forficata* and/or haloperidol-induced on glucose levels. Values are presented as means±S.E.M.

Figure 3: Prevalence of glucose levels higher and lower than 200 mg/dL in rats under long-term treatment with haloperidol and/or *B. forficata.*

Table 1: Effects of long-term treatment with haloperidol and/or *B. forficata* on brain, liver and kidney weights and on brain- liver- and kidney-to-bodyweight ratios. Data are expressed as mean±S.E.M.

Table 2: Plasmatic levels of biochemical parameters. Data are expressed as mean±S.E.M.

Figure 1:

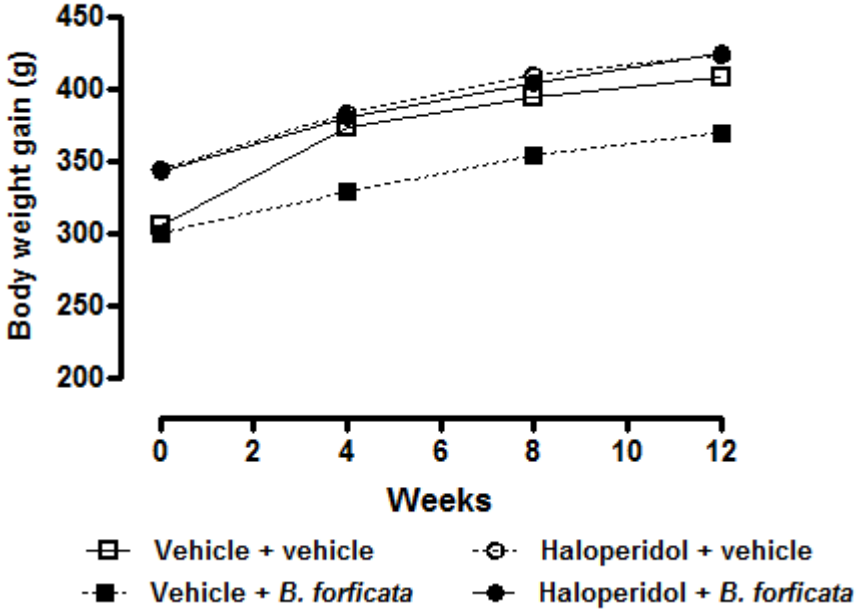
Body weight gain (g)
450
400
350
300
250
200
0
2
4
6
8
10
12
Weeks
Vehicle + vehicle
Haloperidol + vehicle
Vehicle + B. forficata
Haloperidol + B. forficata

Figure 2:

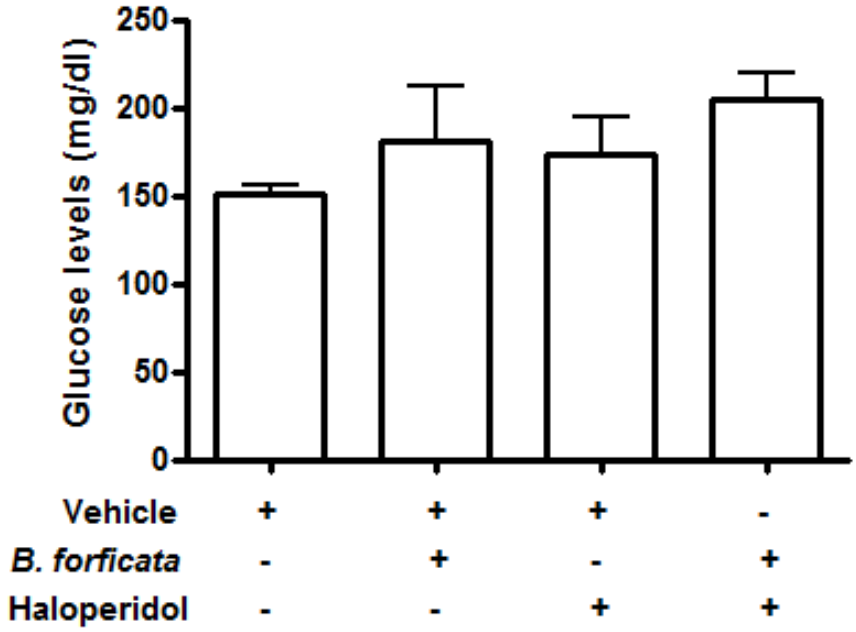
Glucose levels (mg/dl)
250
200
150
100
50
0
Vehicle + + + -
B. forficata - + - +
Haloperidol - - + +

Figure 3:

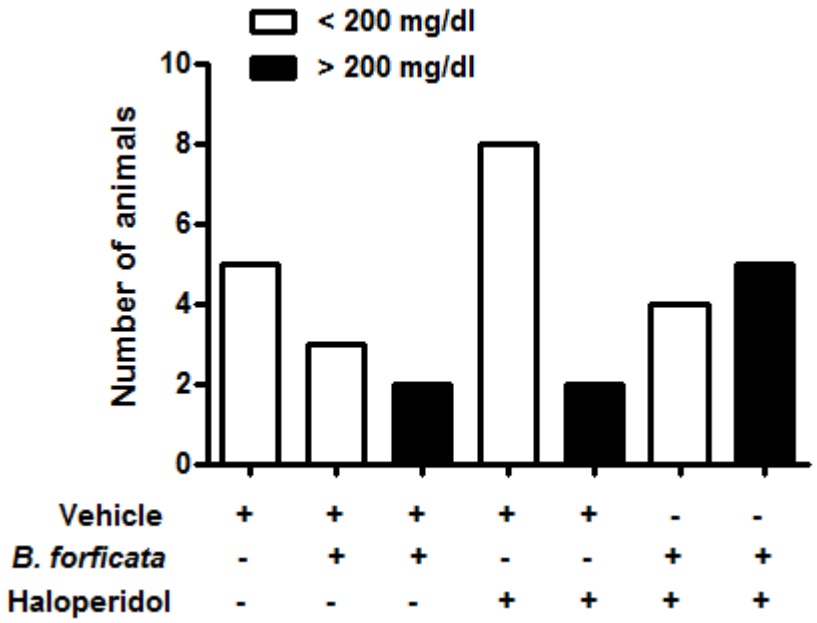
< 200 mg/dl
> 200 mg/dl
Number of animals
10
8
6
4
2
0
Vehicle + + + + + - -
B. forficata - + + - - + +
Haloperidol - - - + + + +

Table 1

Groups	Brain (g)	Brain/body (g/g)%	Liver (g)	Liver/body (g/g)%	Kidney (g)	Kidney/body (g/g)%
Control	1.86±0.04	0.47±0.03	10.20±0.58	2.52±0.14	2.89±0.10	0.69±0.05
B. forficata	1.79±0.07	0.51±0.04	8.78±0.62	2.44±0.09	2.55±0.23	0.71±0.05
Haloperidol	1.78±0.03	0.42±0.01*	9.33±0.36	2.23±0.05	2.63±0.13	0.63±0.03
Haloperidol + *B. forficata*	1.79±0.03	0.42±0.01*	10.29±0.63	2.43±0.14	2.74±0.07	0.66±0.02

* Significant difference from *B. forficata* group.

Table 2

Groups	Creatinine (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	ALT (U/L)	AST (U/L)	Urea (mg/dL)	LDH (U/L)
Control	0.63±0.05	80.40±6.48	53.54±2.43	46.48±4.98	103.20±4.35	17.79±3.62	271.50±45.47
B. forficata	0.67±0.05	72.76±12.24	50.76±6.04	56.39±14.43	91.66±5.72	17.72±4.59	173.10±30.30
Haloperidol	0.66±0.04	76.41±5.34	67.97±6.40	41.61±4.87	98.12±7.10	12.02±1.69	187.60±42.10
Haloperidol + *B. forficata*	0.62±0.06	81.43±7.12	82.64±5.23*	40.64±4.28	100.60±6.25	13.18±2.33	196.00±22.31

* Significant difference from control and *B. forficata* group.

4 DISCUSSÃO

Produtos naturais têm sido empregados em diversos estudos devido às suas propriedades farmacológicas, baixa toxicidade e grande aceitabilidade da população. Tendo em vista isso, o presente estudo avaliou os efeitos da *B. forficata,* uma planta utilizada popularmente para diminuir a glicemia e que possui altas concentrações de compostos antioxidantes, na peroxidação lipídica cerebral *in vitro* induzida por pró-oxidantes, na discinesia orofacial e nas possíveis alterações metabólicas induzidas pelo tratamento crônico com haloperidol em ratos.

A peroxidação lipídica é um processo induzido por radicais livres que leva à oxidação de lipídios poliinsaturados. Isso altera a fluidez de membranas biológicas, levando à disfunção celular (SCHEID et al, 1996). Os resultados do presente estudo mostram que o decocto de *B. forficata* protegeu contra peroxidação lipídica induzida pelos agentes pró-oxidantes, nitroprussiato de sódio (NPS), o complexo Fe^{2+} /EDTA, e Fe^{2+} em homogenatos de cérebro. Esses resultados sugerem que o decocto apresenta grande atividade antioxidante cerebral.

De fato, este estudo observou que o decocto foi mais efetivo contra NPS, do que o complexo Fe^{2+}/EDTA, e Fe^{2+}. Quando o NPS foi utilizado para induzir a lipoperoxidação cerebral, baixa concentração de *B. forficata* (25 μg/ml) foi necessária para reduzir os níveis de lipoperoxidação aos níveis basais, porém quando utilizado Fe^{2+} ou Fe^{2+}/EDTA, foi preciso uma concentração maior (250 μg/ml) para alcançar o mesmo efeito. A neurotoxicidade causada pelo NPS, provavelmente se deve a sua habilidade em gerar radicais $^{\cdot}OH$ via reação de Fenton, catalisado na sua porção de ferro (RAUHALA et al, 1998). O ferro e o complexo Fe^{2+}/EDTA apresentam propriedades pró-oxidantes semelhantes ao NPS, como a geração de radicais $^{\cdot}OH$ que levam à peroxidação lipídica (HALLIWELL e GUTTERIDGE, 1989; DEAN et al., 1997; RAUHALA et al., 1998).

O haloperidol bloqueia RD2 tanto pré-sinápticos, quanto pós-sinápticos no estriado e em outras regiões, o qual causa um efeito compensatório, aumentando a síntese de dopamina, que leva ao aumento da liberação de dopamina e do *turnover* da dopamina (LERNER et al., 1977). Elevados níveis de dopamina e seus metabólitos podem ser oxidados via reação de Fenton, aumentando as concentrações de ERO (ROGOZA et al., 2004), ou metabolizados pela ação da enzima monoamina oxidase (MAO) com formação de H_2O_2 (MARIANI, et al, 2005), o qual pode causar toxicidade cerebral.

O EO já foi proposto como um mecanismo patogênico na DT (CADET et al., 1986; CADET e KAHLER, 1994). Os resultados deste trabalho mostram que o decocto de *B. forficata* foi eficiente em reduzir a peroxidação lipídica cerebral induzida por pró-oxidantes e assim, poderia ser eficiente em reduzir o EO induzido pelo uso de antipsicóticos, atenuando o desenvolvimento da DT.

Busanello et al (2012) mostraram que o tratamento com resveratrol, um polifenol encontrado principalmente em uvas vermelhas e vinhos tinto, reduziu a prevalência de MMV em animais tratados com flufenazina. Em outro estudo, foi demonstrado que a *Ilex paraguariensis* foi capaz de reduzir os MMV e o EO induzido pelo tratamento agudo com haloperidol (COLPO et al. 2007). Por outro lado, Fachinetto et al. 2007b, não detectaram EO no tratamento crônico com haloperidol. Como resultado do presente estudo, observou-se que o tratamento crônico com haloperidol causou prevalência de 50% de DO em ratos tratados com haloperidol (dados não mostrados). Estes dados estão de acordo com (FACHINETTO et al. 2007b). No entanto, não foi observado efeito do decocto de B. *forficata* em reduzir a prevalência dos MMV induzidos pelo haloperidol em ratos. Quanto à intensidade dos MMV, observou-se uma proteção parcial. Esses resultados sugerem que a atividade antioxidante de produtos naturais não necessariamente é a principal razão para sua eficácia em modelo de MMV.

Os efeitos do haloperidol e/ou *B. forficata* na atividade locomotora espontânea em ratos no teste do campo aberto também foram investigados. Este teste é comumente usado para confirmar a eficácia de antagonistas dopaminérgicos. Como esperado, o tratamento com haloperidol diminuiu ambos os parâmetros de locomoção, cruzamentos e levantamentos. A *B. forficata* não foi capaz de evitar essa redução na atividade locomotora. Inesperadamente, *B. forficata* causou um significante aumento no número de cruzamentos, mas não de levantadas. Isso provavelmente aconteceu, pois algum componente da planta estaria agindo em regiões específicas do sistema nervoso central. Sabe-se que a supressão da locomoção por haloperidol, resulta do bloqueio dopaminérgico no núcleo accumbens (KELLEY et al. 1989; SALAMONE et al. 1998). Todavia, o mecanismo envolvido no efeito da *B. forficata* sobre o aumento no número de cruzamentos precisa ser melhor investigado.

No tecido cerebral, o RD2 é expresso nos gânglios basais, núcleo accumbens e no córtex frontal, onde participa do controle extrapiramidal da atividade locomotora (DE MEI et al, 2009). No cérebro, o RD2 é expresso em duas isoformas, a isoforma curta (RD2S– *short*) e a isoforma longa (RD2L – *long*), sendo a RD2L a mais abundante no tecido cerebral (DE MEI

et al, 2009). Além do cérebro, os RD2 estão localizados na retina, rins, pâncreas, sistema vascular e glândula pituitária (MISSALE et al, 1998).

O bloqueio de receptores D1/D2 no núcleo accumbens afeta a atividadade motora, tem pequenos efeitos nos parâmetros alimentares, mas não reduz o consumo de alimento (BALDO et al. 2002). Alguns estudos têm mostrado o envolvimento do RD2 em parâmetros envolvidos no metabolismo. Garcia-Tornadu et al (2010) estudaram a homeostase da glicose em camundongos com expressão reduzida de RD2 (RD2-/-). Os camundongos RD2-/- apresentaram diminuição da resposta da insulina à glicose, alta glicemia em jejum, e foram intolerantes à glicose. A intolerância à glicose resultou de uma diminuição da resposta secretória da insulina, ao invés de resistência à insulina.

O haloperidol que exerce sua função por antagonizar RD2 poderia alterar diferentes parâmetros metabólicos e o uso da *B. forficata*, uma planta popularmente conhecida por apresentar atividade anti-hiperglicêmica, poderia ser eficaz em reduzir os possíveis efeitos colaterais metabólicos causados pelo tratamento crônico com haloperidol.

O co-tratamento com *B. forficata* causou um aumento significativo na glicemia de ratos. A literatura tem sugerido que a *B. forficata* atua como um agente mimético da insulina, aumentando a liberação de insulina das células β pancreáticas. No entanto, o haloperidol pode estar estimulando um aumento na secreção de insulina, algo semelhante encontrado por Baptista et al (2002), onde ratos tratados com um antagonista seletivo de RD2 apresentaram altos níveis de insulina e glicose. Assim, a elevada estimulação de insulina pela *B. forficata* e pelo haloperidol, poderia causar um quadro de resistência à insulina, o qual levaria ao estado hiperglicêmico encontrado neste estudo. Sabe-se que o haloperidol também eleva as concentrações séricas de prolactina. A prolactina aumenta a proliferação de células β, transcrição do gene da insulina e secreção de insulina dependente de glicose em ilhotas pancreáticas isoladas (BRELJE et al, 1993; SORENSON et al, 1993; SORENSON e BRELJE, 1997; PETRYK et al, 2000; FLEENOR e FREEMARK, 2001).

Um estudo *in vitro* mostrou que a kaempferitrina (kaempferol 3,7-dirhamnoside), um flavonóide glicosilado extraído da *Bauhinia acuminata*, inibe a translocação do Transportador de Glicose 4 (GLUT4) estimulada por insulina, também a captação de glicose, através da inativação da Proteína Cinase B (PKB)/Akt que está envolvida na sinalização da translocação do GLUT4, e pelo bloqueio diretamente no canal de transporte da glicose no GLUT4 em adipócitos 3T3-L1 (PRASAD et al, 2009). Outros estudos mostram que o haloperidol modula negativamente a sinalização da Akt mediada por insulina em cultura de neurônios e células

PC12 (UKAI et al., 2004; DAI et al., 2007). Desta forma, outra hipótese para o quadro hiperglicêmico encontrado neste estudo, poderia ser causado devido à presença do kaempferol encontrado na *B. forficata*, o qual estaria impedindo a translocação de GLUT4 no tecido muscular e adiposo, levando à hiperglicemia. Um quadro ainda pior de hiperglicemia foi observado no grupo haloperidol co-tratado com *B. forficata*, pois uma vez que o haloperidol modula negativamente a expressão da Akt na sinalização da insulina, esse efeito poderia ser intensificado pela presença do keampferol presente na *B. forficata.*

Em outro estudo, foi observado que o haloperidol foi capaz de bloquear canais de potássio sensíveis a ATP (K_{ATP}) em células β pancreáticas em roedores, via interação com a subunidade Kir6.2 do canal (YANG et al., 2004). Nas células β pancreáticas, o fechamento de canais de K_{ATP} levam a despolarização da membrana, abrindo canais de cálcio dependentes de voltagem, provocando a liberação de insulina (KANNO et al., 2002). Esse fechamento de canais K_{ATP} causado pelo haloperidol pode ser um mecanismo pelo qual esta droga induz a hiperinsulinemia.

5 CONCLUSÕES ESPECÍFICAS

De acordo com os resultados apresentados nesta dissertação, pode-se concluir:

- a análise do decocto de *B. forficata* por CLAE apresentou grande quantidade de compostos fenólicos, os quais sugerem que a *B. forficata* pode apresentar atividade antioxidante promissora.
- a *B. forficata*, conhecida por sua atividade hipoglicemiante, protegeu contra a peroxidação lipídica cerebral induzida por pró-oxidantes, sugerindo ser uma potente ferramenta contra as injúrias oxidativas cerebrais, e dessa forma, poderia ser um agente benéfico contra as alterações motoras e metabólicas induzidas pelo tratamento com haloperidol em ratos.
- o tratamento crônico com haloperidol causou aumento nos MMV em ratos e a *B. forficata* foi capaz de proteger apenas parcialmente esta alteração motora.
- foi observado no teste do campo aberto que o tratamento com haloperidol causou hipolocomoção, e o co-tratamento com *B. forficata* não alterou esse resultado. Porém, os animais tratados somente com *B. forficata* apresentaram aumento na locomoção.
- as análises bioquímicas, revelaram aumento da glicemia em ratos tratados com haloperidol e o co-tratamento com *B. forficata* exacerbou esse efeito, bem como aumentou os níveis de triglicerídeos. Sugerindo que o tratamento crônico com *B. forficata* pode ser tóxico, levando à

resistência à insulina, uma vez que essa planta apresenta influencias no metabolismo. Não foram observadas alterações significativas nos demais parâmetros metabólicos analisados.
- um significativo aumento de peso foi observado nos ratos tratados com haloperidol, enquanto nenhuma diferença significativa no ganho de peso foi observada nos animais tratados somente com *B. forficata*.

5.1 CONCLUSÃO GERAL

Em conclusão, o primeiro trabalho sugere que o decocto de *B. forficata* apresenta grande potencial antioxidante, pois se mostrou eficiente em reduzir a lipoperoxidação cerebral induzida por pró-oxidantes, porém, não se mostrou eficiente em reduzir completamente os MMV nem a hipolocomoção induzida pelo tratamento crônico com haloperidol em ratos, sugerindo que apesar da capacidade antioxidante, o EO não é principal fator agravante nos distúrbios motores causados pelo tratamento com haloperidol. O segundo trabalho por sua vez, mostrou que o tratamento crônico com haloperidol e/ou *B. forficata* apresentou elevada incidência de hiperglicemia e aumento nos triglicerídeos no grupo haloperidol + *B. forficata*. Os demais parâmetros metabólicos não se alteraram. Todavia, é preciso mais estudos para elucidar o mecanismo de ação desta planta.

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