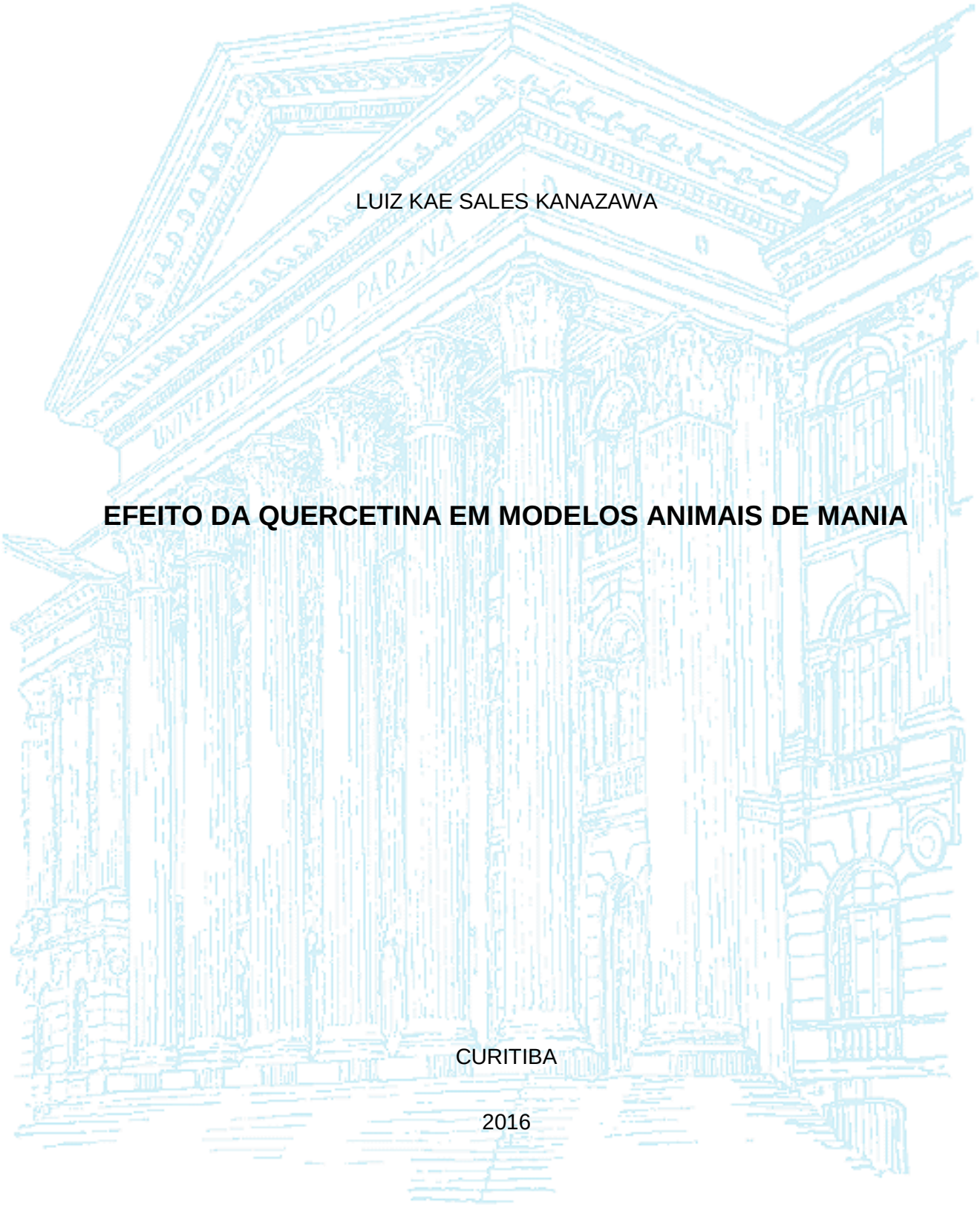
UNIVERSIDADE FEDERAL DO PARANÁ

LUIZ KAE SALES KANAZAWA



EFEITO DA QUERCETINA EM MODELOS ANIMAIS DE MANIA

CURITIBA

2016

LUIZ KAE SALES KANAZAWA

EFEITO DA QUERCETINA EM MODELOS ANIMAIS DE MANIA

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Farmacologia, no Curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientador: Prof. Dr. Roberto Andreatini

CURITIBA

2016

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
Setor CIÊNCIAS BIOLÓGICAS
Programa de Pós Graduação em FARMACOLOGIA
Código CAPES: 40001016038P0

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em FARMACOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da Dissertação de Mestrado de **LUIZ KAE SALES KANAZAWA**, intitulada: **""EFEITO DA QUERCETINA EM MODELOS ANIMAIS DE MANIA"."**, após terem inquirido o aluno e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO.

Curitiba, 24 de Março de 2016.

Prof ROBERTO ANDREATINI (UFPR)
(Presidente da Banca Examinadora)

Prof BRUNO JACSON MARTYNHAK (UFPR)

Prof MARCELO DE MEIRA SANTOS LIMA (UFPR)

AGRADECIMENTOS

Gostaria de agradecer primeiramente a meus pais, **Kazuo** e **Maria Arinda**, por toda a dedicação e apoio dados por toda a minha vida em todos os sentidos. Dia após dia, incessantemente me ajudando, me mantendo, me fortalecendo, me educando... Um dia irei retribuir todo o esforço e toda a luta. Sei o quanto a vida não lhes foi leve nem gentil. Sei também o quanto vocês se esforçaram para que eu não precisasse enfrentar todas as adversidades que vocês enfrentaram sempre. Nada do que conquistei até hoje teria sido possível sem vocês e por isso lhes dedico minha gratidão e amor eternos.

Quero agradecer também à minha família, especialmente meu irmão **Jun** e minha tia **Eliana**, pelo companheirismo, apoio e amizade. Agradeço por todos os momentos engraçados e pela união nos momentos de dificuldade. Vocês são essenciais em minha vida!

Agradeço aos parceiros que me auxiliaram na execução deste projeto. Muito obrigado, **Débora Dalla Vecchia**, **Etiéli Wendler** e **Palloma Hocayen** por todo o tempo e energia gastos por mim! Sou profundamente grato por todo o auxílio, mas também pela amizade, companheirismo e irmandade.

Obrigado **Paulo Sérgio Beirão Jr**. e **Manuela de Mélo** por permitirem que eu desse continuidade ao projeto de vocês! Agradeço de coração a colaboração essencial de **Francislaine Lívero**, **Maria Carolina Stipp**, **Claudia Corso**, **Jonathan Agnes**, **Blanca Gallegos**. Vocês são fantásticos e eu nunca poderei retribuir toda a ajuda que vocês me deram! Muito obrigado pela colaboração, profa. **Alexandra Acco**. Fique ciente da minha gratidão e da minha admiração por você! Agradeço a **Camila Pasquini**, **Sílvia Genaro**, **Andrés Mello**, **Vanessa Benato** e **Vivian Monteiro**, que muito me auxiliaram também!

Agradecimento especial a **Bruno Martynhak**, por ter me ensinado tanto e por ter permitido que eu acompanhasse e auxiliasse em seus projetos de doutorado. Você tem minha admiração e gratidão sempre! Gostaria também de agradecer aos professores que me inspiram e que marcaram a minha vida. Primeiramente, profa. **Maria Fernanda de Paula Werner**, por ter me acolhido em seu laboratório como aluno de iniciação científica, antes mesmo de eu fazer a disciplina de Farmacologia na graduação. Graças a você, eu me apaixonei pela pesquisa científica e me identifiquei com o trabalho em laboratório e pesquisa! Agradeço ao prof. **Aleksander Zampronio** por ter me inspirado com suas aulas de Farmacologia. Com elas, eu me apaixonei por essa área e me fascinei irreversivelmente! Agradeço imensamente ao prof. Dr. **Roberto Andreatini**, meu orientador, que me aceitou como aluno de iniciação científica e posteriormente como aluno de mestrado. Jamais irei me esquecer da aula de depressão e de como soube, naquela aula, que eu deveria seguir a área de Neurociências. Agradeço profundamente as oportunidades que foram me dadas, todo o apoio, orientação, paciência, ensinamentos e principalmente por sempre, mesmo que inconscientemente, me inspirar a continuar nessa área. Agradeço também a minha amiga e profa. **Débora Dalla Vecchia**, que me mostrou o que estava faltando para eu me encontrar na área. Além da paixão pela pesquisa científica, pela Farmacologia e por Neurociências, faltava um pequeno estímulo para a Docência. Acompanhar sua evolução na parte de docência foi um privilégio para mim. Todas as suas dicas e ajuda, todas as suas aulas e todo o processo de ver você administrando turmas de graduação me inspiraram a seguir nessa área! Além disso, gostaria de agradecer à amizade incondicional, todo o apoio nas horas difíceis e toda a parceria nos momentos bons! Jamais poderei retribuir tudo o que você fez por mim!

Agradeço também o companheirismo e parceria dos Representantes Discentes **Débora Dalla Vecchia**, **Bruna Mariana**, **Adriano Targa** e **Ana Noseda**. Jamais irei esquecer os momentos divertidos e todo o trabalho que tivemos juntos nesse um ano que pude exercer a função de representante

discente. Foi um privilégio trabalhar e estar com vocês nesse período! Mais do que experiência, a representação me trouxe pessoas maravilhosas, que se tornaram amigos fantásticos! Vocês são incríveis!

Muito obrigado aos colegas e amigos do Departamento de Farmacologia! Agradeço também aos meus amigos **Maria Fernanda Shimabukuro**, **Ana Letícia Prette Bazoli**, **Marianna Monteiro**, **Aline Bonetti**, **Fernando Bortolozzi** e **Thiago Zilli** pela amizade incondicional, pelo apoio e suporte nos meus momentos mais difíceis, por todos os momentos bons e por estarem sempre comigo! Agradeço as risadas, o carinho, a ajuda, a confiança e agradeço por compartilharem comigo momentos ótimos e momentos não tão ótimos assim! Agradeço a todos que de alguma forma me auxiliaram não só no projeto, no mestrado ou na parte acadêmica, mas também na parte pessoal e emocional.

Por fim, um agradecimento especial ao meu falecido pai, **Kazuo**, o homem mais inteligente que eu já conheci, sem comparação. O homem que me inspirou a sempre estudar, sempre buscar progredir, sempre sonhar alto e ao mesmo tempo me preparar e me qualificar para merecer alcançar meus objetivos. Infelizmente, o falecimento ocorreu alguns meses antes do término do mestrado. Porém, isso só me deu mais força para continuar até o fim e fazer jus à minha promessa de deixar orgulhoso aquele que mesmo em seus últimos dias de vida, mesmo quando o ato de falar exigia um esforço imenso, ainda assim perguntava e se preocupava com meu mestrado. Obrigado, pai.

NOTA EXPLICATIVA

Esta dissertação é apresentada em formato alternativo – artigo para publicação – de acordo com as normas do Programa de Pós-Graduação em Farmacologia da Universidade Federal do Paraná.

RESUMO

O tratamento atual para a mania do transtorno bipolar (TB) envolve grande ocorrência de efeitos adversos, além de altos índices de refratariedade e recaídas, o que torna necessária a pesquisa de novos agentes com potencial antimaníaco. Assim como o lítio, tratamento de primeira linha para a mania no TB, o flavonoide quercetina também possui atividade inibitória sobre a proteína quinase C (PKC) e ação antioxidante. Por isso, a quercetina poderia apresentar potencial antimaníaco. Para investigar este possível efeito, foram avaliados os efeitos do tratamento com quercetina nos modelos de mania de hiperlocomoção induzida por privação de sono paradoxal (PSP) (24 h) e hiperlocomoção induzida por metilfenidato em camundongos. Além disso, foi avaliado o efeito da quercetina no estresse oxidativo, tendo sido analisados os níveis de glutationa reduzida (GSH) e peroxidação lipídica (LPO) no cortex prefrontal (CPF), hipocampo e estriado dos camundongos submetidos aos modelos de mania mencionados. O tratamento agudo (10 e 40 mg/kg) e crônico (10 e 40 mg/kg) com quercetina bloqueou a hiperlocomoção induzida por privação de sono e por metilfenidato, respectivamente. A quercetina também bloqueou a diminuição de GSH no CPF e o aumento de LPO no CPF, hipocampo e estriado de camundongos submetidos à PSP (24 h). O tratamento crônico com quercetina reverteu o aumento de LPO no CPF e no estriado de camundongos submetidos ao modelo de hiperlocomoção induzida por metilfenidato. Tais efeitos foram, no geral, semelhantes ao do lítio, utilizado como controle positivo. Estes resultados indicam um possível efeito tipo antimaníaco da quercetina, o que a torna um agente interessante no contexto da pesquisa de novas drogas antimaníacas.

Palavras-chave: estresse oxidativo, hiperlocomoção, mania, metilfenidato, privação de sono, proteína quinase C (PKC), quercetina, transtorno bipolar (TB).

ABSTRACT

The current treatment for mania in bipolar disorder (BD) involves the occurance of important adverse effects, as well as high rates of refractoriness and relapses, which makes it necessary to research for new potential antimanic agents. Like lithium, the first-line treatment for mania in BD, the flavonoid quercetin also possesses inhibitory activity over protein kinase C (PKC) and antioxidant effects. Thus, quercetin might have an antimanic property. In order to investigate this possible effect, we evaluated the treatment with quercetin in models of mania, such as 24 h paradoxical sleep deprivation (PSD)- and methylphenidate-induced hyperlocomotion in mice. In addition, we evaluated the effect of quercetin on oxidative stress by analyzing the levels of reduced glutathione (GSH) and lipid peroxidation (LPO) in the prefrontal cortex (PFC), hippocampus and striatum of mice that were submitted to the models of mania previously mentioned. Acute (10 and 40 mg/kg) and chronic (10 and 40 mg/kg) treatment with quercetin blocked sleep deprivation- and methylphenidate-induced hyperlocomotion, respectively. Quercetin also blocked the GSH decrease in the PFC and LPO increase in the PFC, hippocampus and striatum of mice submitted to PSD (24 h). Chronic treatment with quercetin reversed the LPO increase in PFC and striatum of mice submitted to the methylphenidate-induced hyperlocomotion model. Those effects were, in general, similar to the effects of lithium, which was used as positive control. These results indicate a possible antimanic-like effect of quercetin, which makes it an interesting agent regarding the research for new antimanic drugs.

Key-words: bipolar disorder (BD), hyperlocomotion, mania, methylphenidate, oxidative stress, protein kinase C (PKC), quercetin, sleep deprivation.

SUMÁRIO

1 INTRODUÇÃO

1.1 TRANSTORNO BIPOLAR

O transtorno bipolar (TB) é uma doença crônica caracterizada por oscilações entre períodos de mania, hipomania e depressão, intercalados com períodos de relativo bem-estar (PRICE; MARZANI-NISSEN, 2012). O TB tem apresentações clínicas complexas, causa prejuízo funcional no paciente e cursa com elevada mortalidade e morbidade (DA SILVA et al., 2011). A prevalência do transtorno bipolar ao longo da vida é estimado em 1% (CASTRO-COSTA; SILVA, 2011). A taxa de mortalidade do TB é de duas a três vezes mais alta comparado às taxas da população geral, sendo que entre 10% e 20% dos pacientes cometem suicídio e cerca de 1/3 deles admitem terem tentado, pelo menos uma vez, cometerem suicídio (MÜLLER-OERLINGHAUSEN et al., 2002).

A fisiopatologia do TB envolve alterações na neurotransmissão dopaminérgica (BERK et al., 2007), glutamatérgica (MICHAEL et al., 2003), serotonérgica (SHIAH; YATHAM, 2000), GABAérgica (BENES; BERETTA, 2001), além de alterações em níveis de segundos mensageiros de vias de sinalização intracelulares (ANDREAZZA et al., 2008), aumento nos níveis plasmáticos de citocinas pró-inflamatórias como IL-6 e TNF-α (BERK et al., 2011), dentre outros fatores.

1.1.1 Mania

Para o diagnóstico de um episódio maníaco do TB, o paciente deve apresentar pelo menos três ou quatro dos seguintes sintomas dentro de um período de pelo menos uma semana de humor elevado: sentimento de grandiosidade, necessidade reduzida de dormir, verborragia, fuga de ideias, distratibilidade, agitação psicomotora e envolvimento em atividades de risco (AMERICAN PSYCHIATRY ASSOCIATION, 2013).

Alguns fatores da fisiopatologia do TB são comuns tanto aos episódios depressivos quanto aos episódios maníacos, como, por exemplo, a diminuição dos níveis de BDNF (ROSA et al., 2014), alterações no funcionamento do eixo hipotálamo-hipófise-adrenal (MANJI; LENOX, 2000) e aumento do estresse oxidativo (BERK et al., 2011; ANDREAZZA et al., 2008). No entanto, alguns fatores como aumento da expressão e da atividade da enzima proteína quinase C (PKC) estão associados aos quadros maníacos (ABRIAL et al., 2015; FRIEDMAN et al., 1993). Durante a fase maníaca do TB, também há aumento da neurotransmissão dopaminérgica (LAHERA et al., 2013), glutamatérgica (MICHAEL et al., 2003), diminuição da neurotransmissão GABAérgica (BENES; BERETTA, 2001), além de maior resposta à serotonina (LÓPEZ-FIGUEROA et al., 2004).

A mania também envolve alterações no ciclo circadiano e no ciclo de sono. Estudos mostram que quadros de insônia parecem ser preditivos de viradas maníacas em pacientes em fase depressiva ou eutimia (FELDMAN-NAIM et al., 1997; PILETZ et al., 1994). Mansell e Pedley (2008) mostraram que pelo menos 75% dos estudos com pacientes bipolares relatam algum tipo de alteração de sono entre os sintomas observados nos pacientes em episódio maníaco. Pacientes maníacos não-medicados apresentam alterações no sono REM como, por exemplo, menor latência para atingir o sono REM e maior densidade de sono REM (HUDSON et al., 1988), que são revertidas por tratamento com lítio (HUDSON et al., 1989). Uma das hipóteses que embasa a relação entre alterações de ciclo circadiano e sono e comportamentos maníacos é a hipótese de que alterações no gene CLOCK possam estar diretamente relacionados a comportamentos similares à mania em humanos, como, por exemplo, hiperatividade, diminuição do sono, diminuição de comportamentos tipo-depressivos, entre outros (ROYBAL et al., 2007).

Diferentemente do que ocorre em relação ao quadro depressivo, não existem tantos modelos animais para avaliação de comportamentos tipo maníacos, o que dificulta a elucidação dos mecanismos envolvidos na fisiopatologia da mania e a pesquisa de novos fármacos para incrementar o

arsenal terapêutico para a mania e o TB (FLAISHER-GRINBERH et al., 2010; EINAT, 2006).

1.1.2 Tratamentos para o TB

Entre os principais tratamentos farmacológicos disponíveis atualmente para o TB, destacam-se os estabilizadores de humor lítio e o valproato de sódio (CHIU et al., 2013; MIKLOWITZ; JOHNSON, 2006), fármacos como a carbamazepina, a lamotrigina (REINARES et al., 2013; MÜLLER-OERLINGHAUSEN et al., 2002) e os antipsicóticos de segunda geração, como a olanzapina, quetiapina, risperidona e aripiprazol (MÜLLER-OERLINGHAUSEN et al., 2002), em esquema de monoterapia, ou associados entre si ou a outras classes de medicamentos, como por exemplo, antidepressivos tricíclicos, antidepressivos inibidores seletivos da recaptação de serotonina (ISRS) e inibidores da MAO (KEMP, 2014; LAFER; NERY, 2011).

Vale ressaltar a importância da pesquisa científica no intuito de encontrar novos agentes terapêuticos para a farmacoterapia do TB, sendo que os tratamentos farmacológicos preconizados para o TB estão relacionados a alguns problemas. A grande ocorrência de efeitos adversos leva a uma queda na adesão terapêutica (CASTRO-COSTA; SILVA, 2011). Dessa forma, mesmo utilizando as mais adequadas estratégias medicamentosas, o curso do transtorno bipolar é caracterizado por altos índices de recorrência de episódios maníacos e depressivos, recaídas e internações (SOUZA, 2011). Além disso, mesmo com a remissão dos episódios de oscilação de humor, ainda podem persistir sintomas subsindrômicos substanciais, principalmente sintomas depressivos, em grande parte dos pacientes (KNAPP; ISOLAN, 2005).

Outro fator importante relacionado à farmacoterapia do TB é o índice de refratariedade. Apenas cerca de 60% dos pacientes com TB respondem ao lítio ou a outros estabilizadores do humor e apenas 40% dos pacientes permanecem sem recaídas/recorrências durante períodos de seguimento de dois a três anos, mesmo em uso de doses adequadas de medicação (KNAPP;

ISOLAN, 2005). Mais de 1/3 dos pacientes com TB não respondem ao tratamento, mesmo sendo ele teoricamente o tratamento adequado (GEDDES; MIKLOWITZ, 2013).

Os medicamentos utilizados na farmacoterapia do TB agem sobre diferentes fatores da fisiopatologia do transtorno, como, por exemplo, o lítio, com seu potencial neuroprotetor, age inibindo a glicogênio sintase quinase 3 (GSK-3), enzima que inibe fatores de transcrição de importantes genes envolvidos em vias de neuroproteção (BERK et al., 2011; GOULD et al., 2006). O lítio também aumenta os níveis de BDNF, aumentando sua expressão gênica (BERK et al., 2011). Outro mecanismo de ação relevante é a ação inibitória do lítio e do valproato sobre a enzima PKC, que está relacionada à ação antimaníaca destes fármacos (WANG; FRIEDMAN, 1989).

1.2 PROTEÍNA QUINASE C (PKC)

A enzima PKC constitui uma família de pelo menos 12 enzimas serina/treonina quinases que estão envolvidas na transdução de sinal de estímulos hormonais, neuroquímicos e de fatores de crescimento (WAY et al., 2000). A ativação de uma variedade de subtipos de receptores acoplados à proteína Gq estimula a PLC, que, sendo ativada, catalisa a conversão de fosfatidilinositol 4,5-bifosfato (PIP_2) em dois segundos mensageiros: o inositol trifosfato (IP_3) e diacilglicerol (DAG). IP_3 estimula a mobilização de íons cálcio intracelulares, enquanto DAG ativa a PKC (MANJI et al., 2001). A PKC ativada, então, migra do citosol para a membrana celular, sendo que a enzima ligada à membrana representa a forma ativa da enzima, capaz de fosforilar seus substratos de forma eficiente (PARKER; MURRAY-RUST, 2004).

A PKC é encontrada em diferentes tipos celulares em diferentes órgãos, estando sua ativação relacionada ao tipo de receptor ativado e ao tipo celular em que se encontra (PARKER; MURRAY-RUST, 2004). A PKC é altamente expressa no sistema nervoso central e a maioria das isoformas da PKC encontradas em diferentes regiões do cérebro, como no hipocampo e córtex

prefrontal (CPF) estão envolvidas na regulação do humor (ABRIAL et al., 2011; WETSEL et al., 1992).

1.2.1 Envolvimento da PKC na mania e no TB

Estudos mostram que pacientes maníacos apresentam maior relação membrana:citosol de PKC em plaquetas, sendo normalizada após tratamento com lítio (FRIEDMAN et al., 1993). Estudos *post-mortem* de cérebros de pacientes com TB mostraram a expressão, atividade e translocação para a membrana aumentadas da PKCγ e PKCζ, quando comparado ao perfil encontrado nos cérebros de indivíduos controle, sem transtornos psiquiátricos (WANG; FRIEDMAN, 1996). Medicamentos utilizados na farmacoterapia do TB, como o lítio e o valproato, são capazes de regular a atividade da PKC. Manji e Lenox, 1999, mostraram que a administração crônica (mas não a aguda) de lítio produz uma redução isoforma-seletiva da PKCα e PKCε no CPF e no hipocampo, sem alterações significativas nas isoformas β, γ, δ, ou ζ. A administração aguda e crônica de anfetamina produz um aumento na atividade da PKC, na relação citosol:membrana, na fosforilação da GAP-43, substrato da PKC, ocasionando alterações a longo prazo na neurotransmissão, que têm relação com o aparecimento de comportamento tipo-maníaco (EINAT et al., 2007).

1.3 ESTRESSE OXIDATIVO

Além da atividade aumentada da enzima PKC, a fisiopatologia da mania também envolve aumento de estresse oxidativo, ocorrendo aumento da produção de radicais livres e depleção de enzimas e moléculas antioxidantes (BERK et al., 2011).

Um dos processos mais envolvidos na geração de radicais livres, espécies reativas de oxigênio (ERO) e espécies reativas de nitrogênio (ERN) é

a fosforilação oxidativa. Dentre as espécies reativas de oxigênio, as fisiologicamente mais relevantes incluem o oxigênio singleto ($^1O_2$), peróxido de hidrogênio (H_2O_2), ozônio (O_3), ácido hipocloroso (HOCl) e os radicais livres ânion superóxido (O^{2-}) e radical hidroxil (OH^-). Alguns exemplos de ERN incluem o óxido nítrico (NO) e o radical livre peroxinitrito ($ONOO^-$) (KOHEN; NYSKA, 2002). EROs e ERNs são capazes de reagir com praticamente todas as biomoléculas, incluindo DNA, RNA, proteínas, carboidratos e lipídios, causando danos nestas moléculas (DIPLOCK et al., 1998).

Estudos mostram aumento consistente de peroxidação lipídica (LPO) por radicais livres e EROs, e alterações em enzimas antioxidantes em pacientes com TB, reforçando a hipótese de que o estresse oxidativo está envolvido na fisiopatologia do TB (BERK et al., 2011; OZCAN et al., 2004).

O sistema de defesa antioxidante endógeno protege as células dos danos causados por radicais livres, EROs e ERNs e outras espécies reativas. Uma das moléculas antioxidantes mais importantes na prevenção de danos oxidativos é a glutationa reduzida (GSH), que é capaz de reduzir proteínas com grupos sulfidril oxidados e também de reduzir os níveis de superóxido e de peróxido de hidrogênio em ação cooperativa com outras enzimas como a glutationa peroxidase (GPx), glutationa redutase (GR), glutationa-S-transferase (GST) e catalase (CAT) (ROSA et al., 2014).

1.3.1 Estresse oxidativo na mania e no TB

Estudos mostram que há alterações nos níveis de enzimas antioxidantes na mania e no TB. A atividade da enzima GR e GST encontram-se aumentadas em pacientes com TB em estágio avançado comparado a pacientes com TB em estágios iniciais (ANDREAZZA et al., 2009). Além disso, estudos mostram que os níveis de LPO encontram-se aumentados em pacientes com TB, independente da fase da doença (ANDREAZZA et al., 2007; MACHADO-VIEIRA et al., 2007).

Vários estudos pré-clínicos corroboram os resultados encontrados em humanos. Brüning et al. (2012), em um modelo animal de mania, mostraram correlação positiva entre a hiperlocomoção e o aumento de níveis de LPO em ratos. No entanto, dentre os estudos pré-clínicos, existe grande variabilidade entre os resultados relativos aos níveis de estresse oxidativo em comportamento tipo maníaco. Níveis de enzimas e moléculas antioxidantes no cérebro e o efeito de drogas com efeito antioxidante variam dependendo da região cerebral, do esquema de tratamento e da droga em questão (JORNADA et al., 2011; BHALLA; DHAWAN, 2009; FREY et al., 2006)

1.4 MODELOS ANIMAIS DE MANIA

Os modelos animais de mania mais frequentemente empregados focam em um aspecto específico do transtorno: a hiperatividade em ratos e/ou camundongos. Isso se deve ao fato de que a hiperatividade pode ser facilmente induzida utilizando psicoestimulantes, por exemplo, e também facilmente mensurada, utilizando equipamentos automatizados de monitores de atividade locomotora, por exemplo (EINAT, 2006).

Métodos não-farmacológicos também podem ser empregados para a indução de comportamento tipo maníaco. Apesar de serem métodos menos rápidos para induzir comportamentos tipo maníacos, tais modelos, como a privação de sono ou manipulações genéticas, têm como vantagem a não ocorrência de interações farmacocinéticas entre psicoestimulantes e a droga teste, por exemplo (PEREIRA, 2013; EINAT, 2006).

Contudo, ainda não existe nenhum modelo animal com excelente validade para mania, pois os modelos mimetizam apenas certas características dos comportamentos maníacos. Além disso, não há nenhum modelo que simule as oscilações de humor características do TB.

1.4.1 Hiperlocomoção induzida por psicoestimulantes

Substâncias psicoestimulantes como a anfetamina e o metilfenidato induzem hiperlocomoção por aumentarem a neurotransmissão dopaminérgica, por estimularem a liberação de dopamina e por bloquearem o transportador de dopamina (DAT), impedindo sua recaptação (O'NEILL; SHAW, 1999). O metilfenidato se liga ao DAT, e em menor grau ao transportador de noradrenalina (NET), inibindo a receptação de dopamina e de noradrenalina nos terminais pré-sinápticos, possibilitando maior ativação dos respectivos receptores (GATLEY et al., 1996; SULZER et al., 1995). A dopamina possui um papel importante na locomoção, sendo que a hiperlocomoção, um sintoma relacionado ao quadro de mania, está relacionada a maior ativação do sistema dopaminérgico (D'AQUILA et al., 2000; DI CHIARA, 1995).

Diversos estudos utilizam a anfetamina (O'NEILL; SHAW, 1999) ou o metilfenidato (BARBOSA et al., 2011) como indutores de hiperlocomoção relacionada a um comportamento tipo maníaco. Como modelo animal de mania, a indução de hiperlocomoção por psicoestimulantes ganhou validade de conteúdo, pois a hiperatividade representa um comportamento importante no quadro de mania (YOUNG et al., 2007) além da administração de psicoestimulantes poder acarretar em um quadro maníaco em pacientes susceptíveis (ANAND et al., 2000). O modelo também apresenta validade preditiva, pois estudos clínicos (VAN KAMMEN; MURPHY, 1975) e pré-clínicos (GOULD et al., 2007; FREY et al., 2006) mostram que o lítio é capaz de reverter as alterações comportamentais induzidas por psicoestimulantes.

1.4.2 Hiperlocomoção induzida por privação de sono

Evidências sugerem que o TB esteja relacionado a distúrbios do ciclo circadiano e alterações de sono (HARVEY, 2008). De acordo com o Manual Diagnóstic e Estatístico V (DSM-V), um dos critérios para o diagnóstico de

mania é a redução da necessidade de dormir (AMERICAN PSYCHIATRY ASSOCIATION, 2013). Serretti e Olgiati (2005) mostraram que 99% dos pacientes com TB em episódios maníacos relataram necessidade reduzida de dormir. Hudson e cols. (1988), através de polissonografia, mostraram que pacientes em episódios maníacos apresentaram redução no tempo total de sono, aumento da atividade no sono REM e maior tempo em vigília, especialmente nas últimas duas horas, durante o período do exame. Estudos clínicos mostram que a privação de sono é capaz de induzir a passagem de um episódio depressivo para um episódio maníaco ou hipomaníaco em pacientes com TB (WEHR et al., 1982)

Métodos ambientais/comportamentais podem ser usados para induzir hiperatividade no contexto de mania em animais. Por exemplo, Gessa e colaboradores (1995) mostram o modelo da privação de sono paradoxal, no qual o animal permanece em plataformas rodeadas de água e com o relaxamento muscular que ocorre no sono REM, o animal cai na água e desperta, interrompendo o sono. Isto resulta em um período curto de hiperatividade que é revertido por tratamento com lítio (desta forma, validando o método de triagem de novas drogas antimaníacas), corroborando o observado na clínica, onde a privação de sono pode precipitar episódios maníacos em pacientes com TB (COLOMBO et al., 1999).

O modelo animal de privação de sono paradoxal está relacionado com várias alterações neuroquímicas, como um down-regulation da expressão da enzima tirosina hidroxilase na substância negra pars compacta, diminuição da neurotransmissão dopaminérgica na substância negra pars compacta e no estriado (LIMA et al., 2012), aumento da expressão de receptores D_2 no estriado (LIMA et al., 2007), além de uma supersensibilidade nos receptores dopaminérgicos (TUFIK et al., 1978). Ressalta-se que os bloqueadores dopaminérgicos são agentes inclusos na farmacoterapia da mania aguda (GESSA et al. 1995). O modelo também reproduz aspectos da psicopatologia do episódio maníaco, mimetizando a hiperatividade, a hipersexualidade e a agressividade dos pacientes maníacos (PEREIRA, 2013), Além disso, por ser

um modelo não-farmacológico, para a privação de sono não é necessário a utilização de drogas, evitando a questão de interação farmacocinética entre a droga indutora do comportamento tipo maníaco e a droga teste, o que pode interferir nos resultados do experimento (PEREIRA, 2013).

Sugere-se que sejam empregados tanto modelos farmacológicos e não-farmacológicos para indução de comportamento tipo maníaco em animais (EINAT, 2006) para melhor embasar o estudo do efeito de drogas testes com potencial antimaníaco.

1.5 QUERCETINA

No contexto da pesquisa por agentes que possam incrementar o arsenal terapêutico para o TB, a quercetina, por apresentar determinadas propriedades biológicas a serem citadas a seguir, poderia apresentar um potencial antimaníaco.

A quercetina (Fig. 1) (do Latim, *quercetum*, ou, floresta de carvalho) é o flavonóide mais amplamente distribuído na natureza. Este flavonóide pertence à classe dos flavonóis e é encontrado em plantas na forma de aglicona ou na forma glicosilada (LAKHANPAL; RAI, 2007).

Apesar de sua ação antioxidante ser seu papel farmacológico mais estudado e compreendido, a quercetina certamente possui vários outros efeitos ainda sendo estudados. Postula-se que muitos destes efeitos sejam decorrentes de sua interação com proteínas em cascatas de sinalização intracelulares, como por exemplo a proteína quinase B (Akt), proteína quinase ativada por mitógeno (MAPK), a fosfatidilinositol 3-quinase (PI3K) e a proteína quinase C (PKC) (DAJAS, 2012; WILLIAMS et al., 2004; AGULLO et al., 1997).

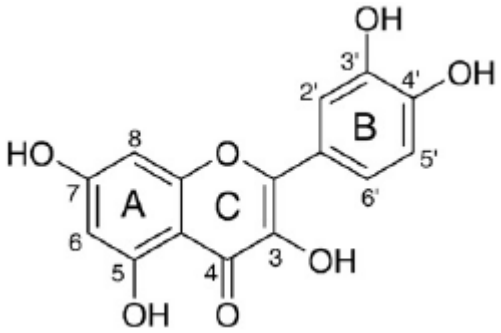
1.5.1 Propriedades da Quercetina

A propriedade da quercetina melhor descrita na literatura é sua ação como antioxidante. A quercetina aparenta ser o flavonóide mais poderoso na

proteção contra espécies reativas de oxigênio produzidas durante o metabolismo normal ou induzidas diante de algum dano (DE GROOT, 1994).

A literatura científica traz vários estudos demonstrando outros efeitos farmacológicos da quercetina, como por exemplo seu efeito antinociceptivo (FILHO et al., 2008), anti-inflamatório (COMALADA et al., 2005), ansiolítico e antidepressivo (BHUTADA et al., 2010), neuroprotetor (DAJAS et al., 2015), antiproliferativo (RUSSO et al., 2012), antibacteriano (RIGANO et al., 2007), antiulcerativo (DE LA LASTRA et al., 1994), dentre outros.

FIGURA 1 – ESTRUTURA QUÍMICA DA QUERCETINA



Fonte: Boots, Haenen e Bast (2008).

Levando em consideração que a fisiopatologia da mania e do TB envolve aumento de estresse oxidativo e aumento da atividade da enzima PKC e que a quercetina apresenta efeito antioxidante e efeito inibitório sobre a PKC, este flavonoide poderia apresentar um efeito tipo antimaníaco, assim como o lítio, que atua nessas duas vias e que é uma droga já empregada na clínica na farmacoterapia da mania e do TB.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar os efeitos da administração de quercetina no comportamento tipo maníaco e no estresse oxidativo no córtex pré-frontal, hipocampo e estriado de camundongos submetidos ao modelo de mania de indução de hiperlocomoção por privação de sono paradoxal e por metilfenidato.

2.2 OBJETIVOS ESPECÍFICOS

Avaliar o efeito da administração de quercetina na hiperlocomoção induzida por privação de sono de 24 h em camundongos (Artigo 1)

Avaliar o efeito da administração de quercetina no estresse oxidativo induzido por privação de sono de 24 h através da análise dos níveis de GSH e LPO no CPF, hipocampo e estriado de camundongos (Artigo 1)

Avaliar o efeito da administração aguda e crônica de quercetina na hiperlocomoção induzida por metilfenidato em camundongos (Artigo 2)

Avaliar o efeito da administração aguda e crônica de quercetina no estresse oxidativo induzido por metilfenidato através da análise dos níveis de GSH e LPO no CPF, hipocampo e estriado de camundongos (Artigo 2)

Verificar possível correlação entre o comportamento tipo maníaco (hiperlocomoção) e as alterações nos parâmetros de estresse oxidativo (Artigos 1 e 2)

3. ARTIGO 1

Quercetin reduces manic-like behavior and brain oxidative stress induced by paradoxical sleep deprivation in mice

Luiz K. S. Kanazawa,[a] Débora D. Vecchia,[a] Etiéli M. Wendler,[a] Palloma de A. S. Hocayen,[a] Francislaine A. dos Reis Lívero,[b] Maria Carolina Stipp,[b] Inara M. R. Barcaro,[a] Alexandra Acco,[b] Roberto Andreatini[a,] *

[a] Laboratory of Physiology and Pharmacology of the Central Nervous System, Department of Pharmacology, Federal University of Paraná, Centro Politécnico, 81540-990, Curitiba, PR, Brazil

[b] Laboratory of Pharmacology and Metabolism, Department of Pharmacology, Federal University of Paraná, Centro Politécnico, 81540-990, Curitiba, PR, Brazil

*Corresponding author

ABSTRACT

Quercetin is a known antioxidant and protein kinase C (PKC) inhibitor. We hypothesized that quercetin affects manic symptoms. Previous studies have shown that mania involves oxidative stress and an increase in PKC activity. In the present study, manic-like behavior (hyperlocomotion) and oxidative stress were induced by 24 h paradoxical sleep deprivation (PSD) in male Swiss mice. Both 10 and 40 mg/kg quercetin prevented PSD-induced hyperlocomotion. Quercetin reversed the PSD-induced decrease in glutathione (GSH) levels in the prefrontal cortex (PFC) and striatum. Quercetin also reversed the PSD-induced increase in lipid peroxidation (LPO) in the PFC, hippocampus, and striatum. Pearson's correlation analysis revealed a negative correlation between locomotor activity and GSH in the PFC in sleep-deprived mice and a positive correlation between locomotor activity and LPO in the PFC and striatum in sleep-deprived mice. These results suggest that quercetin exerts an antimanic-like effect at doses that do not impair spontaneous locomotor activity, and the antioxidant action of quercetin might contribute to its antimanic-like effects.

Key-words: Bipolar disorder, mania, oxidative stress, paradoxical sleep deprivation, protein kinase C, quercetin.

Abbreviations: BD, bipolar disorder; CAT, catalase; CMC, carboxymethylcellulose; CNS, central nervous system; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); GPx, glutathione peroxidase; GSH, reduced glutathione; GSK-3, glycogen synthase kinase-3; LPO, lipid peroxidation; PFC, prefrontal cortex; PKC, protein kinase C; PSD, paradoxical sleep deprivation; SOD, superoxide dismutase.

1. Introduction

Bipolar disorder (BD) is a chronic disease that is characterized by recurrent episodes of depression and mania [1]. It is a common, disabling, and recurrent mental health condition that has various levels of severity [2] and prevalence of 1-3% worldwide [3]. Bipolar patients experience both symptoms of the disease and also impaired functioning, compromised quality of life, and stigma [4]. Lithium is the main pharmacological treatment for BD. Other treatments include the anticonvulsants valproate, carbamazepine, and lamotrigine and atypical antipsychotics quetiapine, risperidone, olanzapine, and aripiprazole [5-7]. The currently available treatments for BD have many side effects [2], such as xerostomia, polydipsia, polyuria, weight gain, insulin resistance, extrapyramidal symptoms, and sexual dysfunction, among others [2, 5, 7, 8]. The great number of side effects results in low treatment adherence [6, 9], and more than one-third of BD patients do not respond to treatment, even when it is adequate [10, 11].

The pathophysiology that underlies BD involves alterations in neurotransmitter levels [12, 13], increases in the activity of protein kinase C (PKC) [14], and increases in oxidative stress [15, 16]. Studies have shown that chronic treatment with lithium and valproate at therapeutic concentrations exert robust antioxidant effects *in vitro* by inhibiting glutamate-induced DNA fragmentation, lipid peroxidation (LPO), and protein oxidation [17]. Souza et al. (2014) [18] reported an increase in oxidative stress in rats that exhibited ouabain-induced hyperlocomotion (i.e., an animal model of mania). Lithium also prevented manic-like behavior in rats and the ouabain-induced increase in superoxide dismutase (SOD) activity and decreases in catalase (CAT) and glutathione peroxide (GPx) activity in the cerebral cortex and hippocampus.

Another relevant mechanism of action of lithium is its inhibitory effect on the enzyme PKC, which can be related to its antimanic actions [19]. Wang and Friedman (1996) [20] showed that post-mortem brains of BD patients had increases in the translocation of PKCγ and PKCζ to the membrane, which

indicates higher levels of the active form of these enzymes. Similarly, some of the pharmacological activities of flavonoids, such as the flavonol quercetin, have been postulated to result from inhibitory activity on kinases, such as PKC [21]. However, quercetin is best known for its antioxidant properties. [22,23]. It appears to be one of the most powerful flavonoids with regard to protecting the body against reactive species that are produced during normal oxygen metabolism or induced by exogenous damage [24, 25]. Quercetin is able to increase the levels or activity of antioxidants, such as glutathione (GSH), CAT, and SOD, and decrease in LPO [26].

Manic patients experience sleep disturbances [27], and the model of paradoxical sleep deprivation (PSD) is considered a non-pharmacological animal model of mania that induces manic-like behavior (i.e., hyperlocomotion) [1,27,28,30] and increases in oxidative stress [27, 31].

Considering the relationship between manic symptoms and increases in PKC activity and oxidative stress in BD, the objective of the present study was to evaluate the effects of the antioxidant and PKC inhibitor quercetin on manic-like behavior and oxidative stress in mice that were subjected to PSD-induced hyperlocomotion.

2. Material and methods

2.1. Animals

The experiments were conducted using male Swiss mice (30-40 g) housed at 22°C ± 2°C under a 12 h/12 h light/dark cycle (lights on at 7:00 AM). The animals were kept in polypropylene cages (41 cm × 34 cm × 16 cm) with food and water available *ad libitum*. All of the experiments were approved by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA/BIO-UFPR, protocol no. 733).

2.2. Drugs

The mice were treated with saline (0.9% NaCl; 10 ml/kg, i.p.), lithium carbonate (Eurofarma, Brazil; positive control; 100 mg/kg, i.p., dissolved in saline, with the pH adjusted to 7.4 with HCl), or quercetin (Sigma, St. Louis, MO, USA; 10 or 40 mg/kg, i.p., suspended in 0.5% carboxymethylcellulose [CMC]). All of the drugs were administered in a volume of 10 ml/kg of body weight. The doses were based on data from the literature [32] and previous studies by our research group [33, 34].

2.3. Paradoxical sleep deprivation protocol

Prior to sleep deprivation, basal spontaneous locomotor activity was measured in an automated activity box for 20 min. Based on the animals' basal locomotor activity, they were divided by stratified randomization into sleep-deprived and non-sleep deprived groups and further subdivided into vehicle, lithium, and 10 and 40 mg/kg quercetin groups. The mice were treated with the respective drugs and sleep-deprived for 24 h according to an adaptation of the multiple platform protocol [31].

Briefly, in the sleep deprivation procedure, groups of six animals were placed in polypropylene cages (41 cm × 34 cm × 16 cm). Each cage contained 12 platforms (3 cm diameter, 5 cm height), surrounded by water up to 1 cm below the surface of the platforms. The animals could move freely, jumping from one platform to another. Food and water were available during the entire procedure.

Thirty minutes before the end of the deprivation period (i.e., 23.5 h after beginning the sleep deprivation period), the animals were treated again with the respective drugs. After the deprivation period of 24 h, the animals were immediately placed in the automated activity box for 20 min to evaluate locomotor activity (Fig. 1).

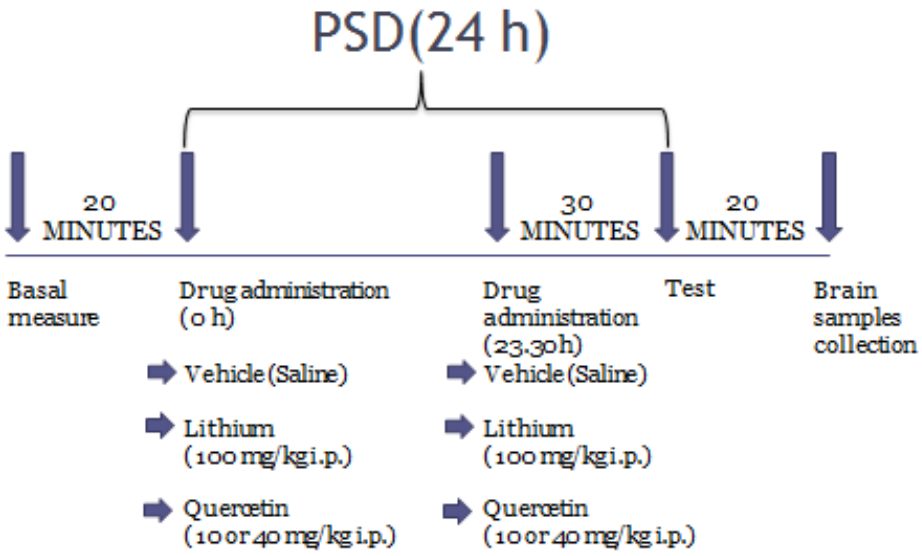


Fig. 1. Timeline of the paradoxical sleep deprivation protocol.

2.4. DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is used to evaluate the free radical scavenging activity of antioxidants. The assay is based on the principle that DPPH, upon accepting a hydrogen atom from a scavenger molecule (e.g., an antioxidant), is reduced, and the purple color of the solution changes to yellow, concomitant with a decrease in absorbance [35].

Following the protocols described by Blois (1958) [36] and Chen et al. (2004) [37], with minor modifications, different concentrations of either quercetin or lithium (1, 3, 10, 30, 100, 300, and 1000 μg/ml) were mixed with DPPH methanolic solution (10 μg/ml). Ascorbic acid (50 μg/ml) was used as a positive control. Distilled water with 0.5% CMC was used as a negative control. Absorbance was measured at 517 nm using a multi-mode microplate reader (BioTek Synergy HT, BioTek Instruments, Winooski, VT, USA).

2.5. Evaluation of oxidative stress parameters in the mouse brain

2.5.1. Brain samples

The mice were euthanized by decapitation immediately after being exposed to the automated activity box. The prefrontal cortex (PFC), hippocampus, and striatum were dissected, frozen in liquid nitrogen, and stored at -80ºC until further analysis. The brain samples were homogenized in potassium phosphate buffer (0.1 M, pH 6.5) in a 1:10 dilution. One part of the homogenate was used to determine GSH levels, and the other was centrifuged at 9000 × *g* in a micro-high-speed refrigerated centrifuge (VS-15000 CFNII, Vision Scientific, Daejeon, South Korea) for 20 min. The supernatant was used to evaluate LPO.

2.5.2. Evaluation of reduced glutathione levels

To evaluate GSH levels, 100 μl of the homogenate was mixed with 80 μl of 12.5% trichloroacetic acid and centrifuged at 6000 rotations per minute (rpm) for 15 min at 4ºC. Afterward, 20 μl of the supernatant was mixed with 280 μl of Tris buffer (0.4 M, pH 8.9) and 5 μl of DTNB (5,5'-dithiobis-[2-nitrobenzoic acid] or Ellman's reagent; 0.01 M) according to the protocol that was originally described by Sedlak and Lindsay (1968) [38], with minor modifications. Absorbance was read at 415 nm using a multi-mode microplate reader (BioTek Synergy HT, BioTek Instruments, Winooski, VT, USA). The individual values were interpolated in a standard curve of GSH (0.375-3 μg) to verify the linearity of the reaction (r^2 must be > 0.99), and the values were divided by a correction factor. The results are expressed as μg/g of tissue, representing the quantity of GSH (μg) in the tissue (g).

2.5.3. Evaluation of lipid peroxidation

Lipid peroxidation was measured according to the method described by Jiang et al. (1992) [39], with minor modifications. First, 100 μl of the supernatant was suspended in 100 μl of methanol, vortexed, and centrifuged at 5000 rpm for 5 min at 4ºC. Afterward, 100 μl of the supernatant was added to 900 μl of FOX2 reagent (Wolff's reagent; 4 mM BHT, 250 μM $FeSO_4$, 250 mM H_2SO_4, and 100 mM xylenol orange). The samples were then vortexed and incubated for 30 min at room temperature in the dark. Absorbance was read at 560 nm using a multi-mode microplate reader (BioTek Synergy HT, BioTek Instruments, Winooski, VT, USA). The results are expressed as mmol of hydroperoxides/mg of tissue.

2.6. Statistical analysis

For all of the experiments, two-way analysis of variance (ANOVA) was used, with the exception of the DPPH assay, followed by the Newman-Keuls *post hoc* test if significant main effects or interactions were found in the ANOVA. The DPPH assay data were analyzed using one-way ANOVA followed by the Newman-Keuls *post hoc* test. The data are expressed as mean ± SEM. Values of $p < 0.05$ were considered statistically significant. Pearson's correlation analysis was performed to identify possible relationships between the behavioral and oxidative stress parameters (all mice, $n = 38\text{-}42$).

3. Results

3.1 Effect of quercetin on 24 h paradoxical sleep deprivation-induced hyperlocomotion

The two-way ANOVA revealed significant main effects of sleep deprivation ($F_{1,50} = 4.211$, $p < 0.05$) and treatment ($F_{3,50} = 19.527$, $p < 0.001$) and a sleep deprivation × treatment interaction ($F_{3,50} = 4.807$, $p < 0.01$). The

Newman-Keuls *post hoc* test revealed that sleep deprivation increased locomotor activity compared with the control group (non-sleep-deprived + vehicle; $p < 0.001$) and all of the other groups (all $p < 0.01$; Fig. 2). Treatment with lithium and 10 and 40 mg/kg quercetin blocked PSD-induced hyperlocomotion ($p < 0.001$). Sleep-deprived mice that were treated with 40 mg/kg quercetin exhibited a decrease in locomotor activity compared with control mice ($p < 0.05$). Non-sleep-deprived mice that were treated with 40 mg/kg quercetin exhibited a tendency toward a decrease in locomotor activity ($p = 0.055$).

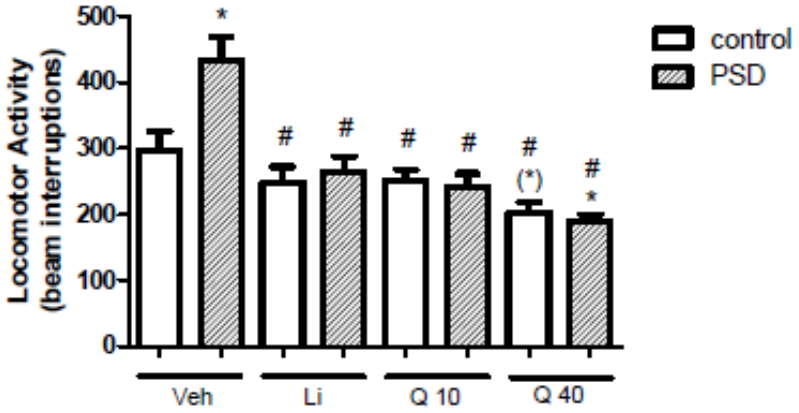


Fig. 2. Effect of acute quercetin (Q; 10 and 40 mg/kg, i.p.) and lithium (Li; 10 mg/kg, i.p.) administration on 24 h PSD-induced hyperlocomotion. Control: non-deprived mice. PSD: sleep-deprived mice. Veh, vehicle. The data are expressed as the mean ± SEM number of beam breaks over 20 min. n = 7-8 mice/group. $^{*}p < 0.05$, (*) $0.05 < p < 0.10$, compared with non-sleep-deprived mice treated with vehicle; $^{\#}p < 0.05$, compared with sleep-deprived mice treated with vehicle (two-way ANOVA followed by Newman-Keuls *post hoc* test).

3.2. Oxidative stress

3.2.1. DPPH assay

The one-way ANOVA of the free radical scavenging activity of lithium and quercetin in the DPPH assay revealed a significant antioxidant effect ($F_{8,18}$ =

137.39, $p < 0.001$, and $F_{8,18} = 52.070$, $p < 0.001$, respectively; Fig. 3A, B). The *post hoc* comparisons showed that all concentrations of lithium, quercetin, and ascorbic acid significantly reduced DPPH levels ($p < 0.001$).

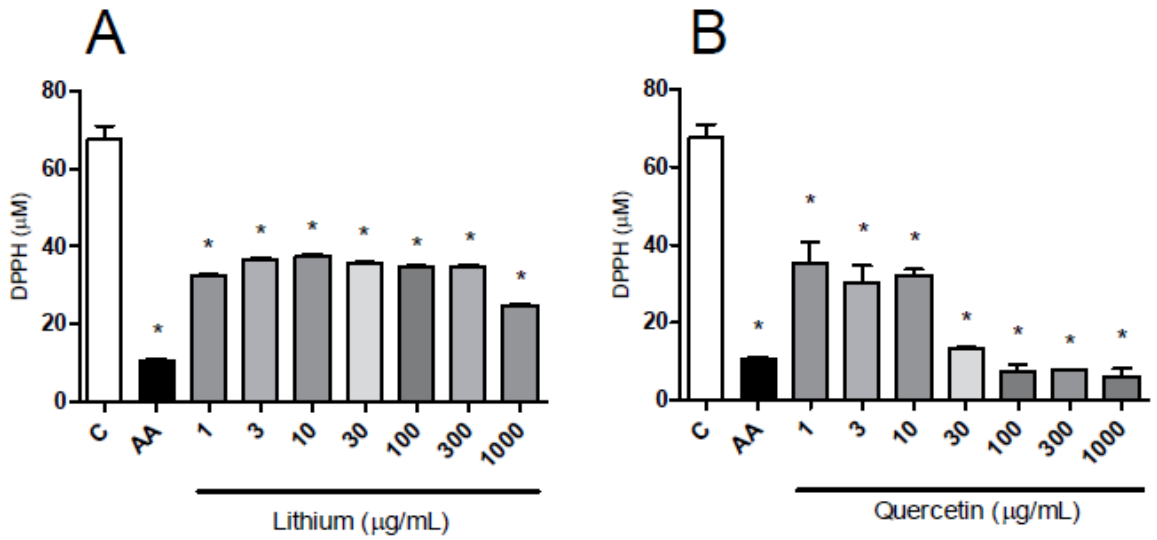


Fig. 3. Effects of lithium (A), quercetin (B), vehicle (C; negative control), and ascorbic acid (AA; positive control) on free radical scavenging activity in the DPPH assay. The data are expressed as mean ± SEM. The tests were performed in triplicate. *$p < 0.001$, compared with control (C).

3.2.2. Effect of acute administration of quercetin on glutathione levels after paradoxical sleep deprivation

The two-way ANOVA revealed a significant effect of treatment ($F_{3,31} = 16.936$, $p < 0.001$) on GSH levels in the PFC and a sleep deprivation × treatment interaction ($F_{3,31} = 12.114$, $p < 0.001$). In the hippocampus, there was a significant effect of treatment ($F_{3,31} = 3.333$, $p < 0.05$) on GSH levels. The two-way ANOVA also revealed significant effects of sleep deprivation ($F_{1,30} = 8.720$, $p < 0.001$) and treatment ($F_{3,30} = 12.262$, $p < 0.001$) on GSH levels in the

striatum and a sleep deprivation × treatment interaction ($F_{3,30}$ = 8.701, p < 0.001). The *post hoc* tests revealed that PSD decreased GSH levels in the PFC (Fig. 4A) and striatum (Fig. 4C) compared with control group (p < 0.05, PFC; p < 0.01, striatum).

Treatment with 10 and 40 mg/kg quercetin and lithium blocked the PSD-induced decrease in GSH levels in the PFC (p < 0.001; Fig. 4A). The *post hoc* comparisons revealed that mice treated with 10 and 40 mg/kg quercetin (non deprived + deprived) exhibited an increase in GSH levels in the hippocampus (Fig. 4B) compared with the vehicle group (non deprived + deprived; p < 0.05). The *post hoc* comparisons revealed that sleep-deprived mice that were treated with 10 mg/kg quercetin exhibited an increase in GSH levels in the striatum compared with the control group (p < 0.001; Fig. 4C). Sleep-deprived mice that were treated with lithium exhibited lower GSH levels in the striatum compared with the control group (p < 0.001) and non-sleep-deprived mice that were treated with lithium (p < 0.05). Sleep-deprived mice that were treated with 40 mg/kg quercetin did not differ from either group that was treated with vehicle (p > 0.05).

3.2.3. Effect of acute administration of quercetin on lipid peroxidation after paradoxical sleep deprivation

The two-way ANOVA revealed significant effects of PSD ($F_{1,34}$ = 44.150, p < 0.001) and treatment ($F_{3,34}$ = 7.922, p < 0.001) on LPO in the PFC and a PSD × treatment interaction ($F_{3,34}$ = 4.534, p < 0.001). In the hippocampus, there were significant effects of sleep deprivation ($F_{1,34}$ = 64.486, p < 0.001) and treatment ($F_{3,34}$ = 6.895, p < 0.001) on LPO. The two-way ANOVA revealed a significant effect of sleep deprivation ($F_{1,30}$ = 25.231, p < 0.001) on LPO in the striatum and a PSD × treatment interaction ($F_{3,30}$ = 9.584, p < 0.001).

Sleep deprivation increased LPO in the PFC ($p < 0.05$), hippocampus ($p < 0.05$), and striatum ($p < 0.05$) compared with the control group. Sleep-deprived mice that were treated with lithium ($p < 0.05$), 10 mg/kg quercetin ($p < 0.001$), and 40 mg/kg quercetin ($p < 0.01$) exhibited a decrease in LPO in the PFC compared with sleep-deprived mice that were treated with vehicle (Fig. 5A). Sleep-deprived mice that were treated with 10 mg/kg quercetin exhibited a significant increase in LPO compared with non-sleep-deprived mice that were treated with 10 mg/kg quercetin ($p < 0.05$). Sleep-deprived mice that were treated with 10 mg/kg quercetin and lithium exhibited an increase in LPO compared with the control group ($p < 0.05$).

The *post hoc* comparisons showed that sleep-deprived mice that were treated with lithium ($p < 0.05$), 10 mg/kg quercetin ($p < 0.001$), and 40 mg/kg quercetin ($p < 0.05$) exhibited a decrease in LPO in the hippocampus compared with sleep-deprived mice (Fig. 5B). Sleep-deprived mice that were treated with lithium and 40 mg/kg quercetin exhibited an increase in LPO compared with the control group ($p < 0.05$) and non-sleep-deprived mice that were treated with the respective drugs ($p < 0.01$).

The *post hoc* comparisons revealed that sleep-deprived mice that were treated with lithium ($p < 0.01$) and 10 mg/kg quercetin ($p < 0.05$) exhibited a decrease in LPO in the striatum compared with sleep-deprived mice that were treated with vehicle (Fig. 5C). Treatment with 40 mg/kg quercetin did not affect the PSD-induced increase in LPO ($p > 0.05$).

3.2.4. Correlation between PSD-induced hyperlocomotion and indices of oxidative stress

The correlation analysis revealed a negative correlation between locomotor activity and GSH levels in the PFC in sleep-deprived mice ($r = -0.76$, $p < 0.001$) and a positive correlation between locomotor activity and LPO in the PFC ($r = 0.53$, $p < 0.05$) and striatum ($r = 0.49$, $p < 0.05$) in sleep-deprived mice (Table 1).

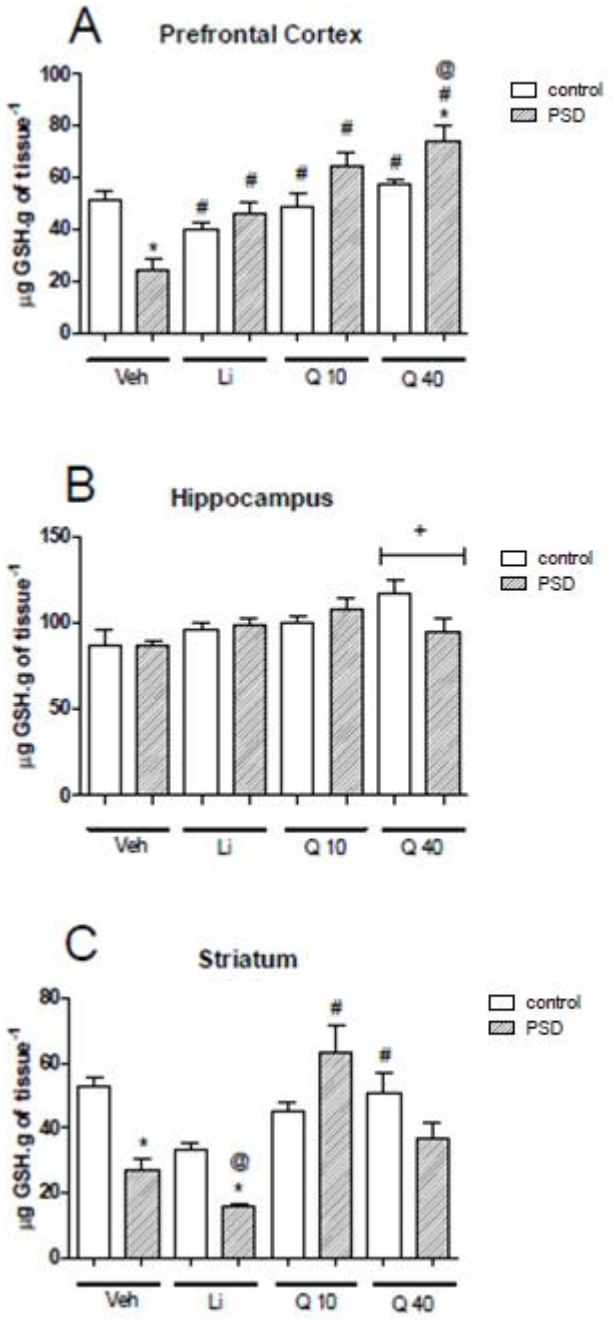


Fig. 4. Effect of acute administration of quercetin (Q; 10 and 40 mg/kg, i.p.) and lithium (Li; 100 mg/kg, i.p.) on GSH levels in the prefrontal cortex (A), hippocampus (B), and striatum (C) in mice subjected or not to 24 h PSD. The data are expressed as mean ± SEM. n = 6 mice/group. $^{*}p < 0.05$, compared with non-sleep-deprived mice treated with vehicle; $^{\#}p < 0.05$, compared with sleep-deprived mice treated with vehicle; $^{@}p < 0.05$, compared with non-sleep-deprived mice treated with the same drug; $^{+}p < 0.05$, compared with sleep-deprived and non-sleep-deprived mice that were treated with vehicle. Control: non-deprived mice. PSD: sleep-deprived mice.

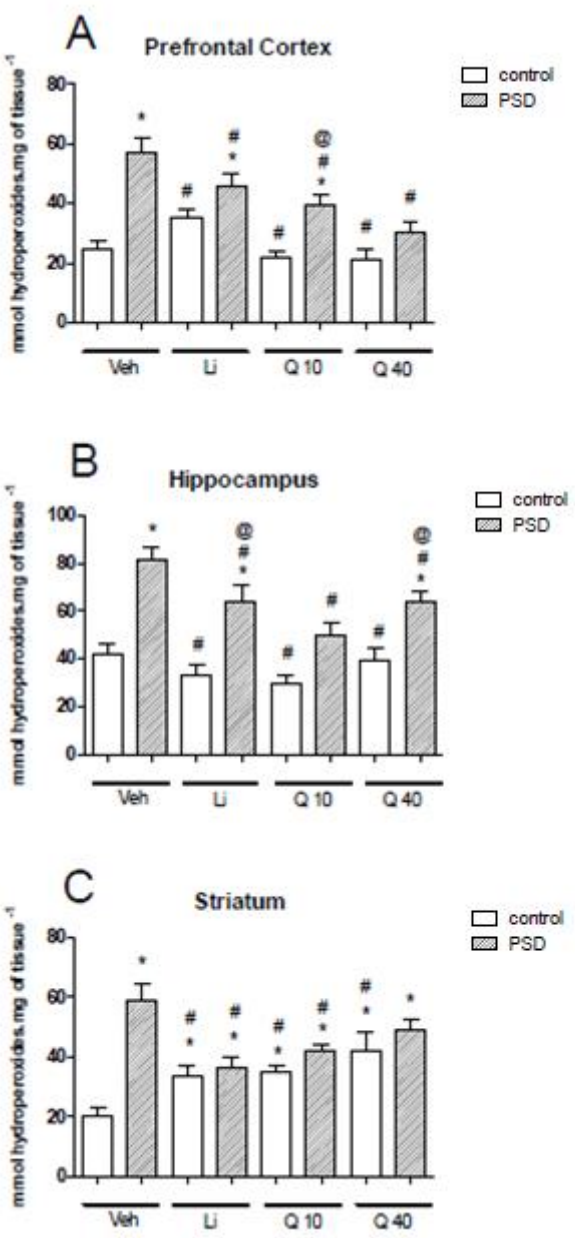


Fig. 5. Effect of acute administration of quercetin (Q; 10 and 40 mg/kg. i.p.) and lithium (Li; 100 mg/kg, i.p.) on LPO in the prefrontal cortex (A), hippocampus (B), and striatum (C) in mice that were or were not subjected to 24 h PSD. The data are expressed as mean ± SEM. n = 6 mice/group. *$p < 0.05$, compared with non-sleep-deprived mice treated with vehicle; $^{\#}p < 0.05$, compared with sleep-deprived mice treated with vehicle; $^{@}p < 0.05$, compared with non-sleep-deprived mice treated with the same drug. Control: non-deprived mice. PSD: sleep-deprived mice.

When considering all of the animals together, a negative correlation was found between locomotor activity and GSH levels (r = -0.57, p < 0.001), and a positive correlation was found between locomotor activity and LPO (r = 0.44, p < 0.01) in the PFC. The correlation analysis also revealed a tendency towards a positive correlation between locomotor activity and LPO in the hippocampus (r = 0.30, 0.05 < p < 0.01) and striatum (r = 0.32, 0.01 < p < 0.05). In the sleep-deprived group, a tendency toward a positive correlation was found between locomotor activity and LPO in the hippocampus (r = 0.39, 0.01 < p < 0.05; Table 1).

Table 1. Correlation coefficients (Pearson's r) between paradoxical sleep deprivation-induced hyperlocomotion and glutathione and lipid peroxidation in the hippocampus, striatum, and prefrontal cortex.

	Hippocampus		**Striatum**		**Prefrontal Cortex**	
Locomotor Activity	*GSH*	*LPO*	*GSH*	*LPO*	*GSH*	*LPO*
All mice (n = 38-42)	-0.14	0.30(*)	-0.14	0.32(*)	-0.57***	0.44**
Sleep-deprived (n = 17-22)	-0.30	0.39(*)	-0.15	0.49*	-0.76***	0.53*
Non-sleep-deprived (n = 19-21)	0.10	0.12	0.04	-0.10	-0.05	0.07

*p < 0.05, **p < 0.01, ***p < 0.001, (*) 0.01 < p < 0.05.

4. Discussion

The present study showed that 24 h sleep-deprived mice exhibited hyperlocomotion compared with control non-sleep-deprived mice. Acute treatment with quercetin at doses that did not impair spontaneous locomotor activity and acute treatment with lithium blocked PSD-induced hyperlocomotion. Thus, our findings demonstrated a potential antimanic-like effect of quercetin, indicating that this flavonoid may be a promising therapeutic agent for the treatment of manic symptoms.

Animal models of mania frequently focus on a specific aspect of the disorder, namely hyperactivity. This feature can be easily induced and

monitored. Non-pharmacological or environmental methods, such as PSD, are widely used to induce manic-like behavior in animals [40]. Gessa et al. (1995) [28] showed that sleep deprivation results in a short period of hyperactivity that is responsive to lithium, which validates the model for screening novel antimanic drugs. In the clinical context, during a manic episode, 69-99% of patients experience a reduced need for sleep [41]. Indeed, many studies have shown that BD patients present sleep disturbances [41,42], and sleep deprivation can predict manic episodes [43]. An important validation of sleep deprivation-induced manic-like symptoms as a model for the human condition is its sensitivity to lithium [28]. In the present study, the PSD model was sensitive to lithium treatment, in which sleep-deprived animals that were treated with lithium exhibited a reduction of hyperlocomotion compared with sleep-deprived animals that were treated with vehicle.

Tufik et al. (1978) [29] showed that sleep deprivation caused a hypersensitivity in dopaminergic receptors. Ghosh et al. (1976) [30] showed that the dysregulation in dopaminergic neurotransmission caused an increase in locomotor activity. In humans, amphetamine, which increases dopaminergic neurotransmission, was shown to increase motor activity in a human open field test [44]. Likewise, the alterations in dopaminergic neurotransmission that are caused by sleep deprivation are a source of oxidative stress in the brain, which might be related to the establishment of manic symptoms [45, 46]. The central nervous system (CNS) is vulnerable to oxidative stress. The brain utilizes great amounts of oxygen, consequently promoting the formation of oxygen free radicals and reactive oxygen species. Corroborating the notion that oxidative stress may indeed be related to the pathophysiology of BD and manic-like behavior, Souza et al. (2014) [18] showed that melatonin treatment decreased ouabain-induced hyperlocomotion in rats. Their study also showed a positive correlation between manic-like behavior and indices of oxidative stress in the PFC and hippocampus.

In the present study, the antioxidant property of quercetin was demonstrated in the DPPH assay (Fig. 3B). Our results also indicated that 10

mg/kg quercetin increased GSH levels in the PFC and striatum, attenuating the PSD-induced decrease in GSH levels in these brain areas. Treatment with 40 mg/kg quercetin blocked the PSD-induced decrease in GSH levels in the PFC. Lithium also exerts neuroprotective effects against oxidative stress [47], which is consistent with our DPPH assay results (Fig. 3A). Our results showed that acute lithium treatment increased GSH levels in the PFC, whereas in the striatum it did not. Previous studies have shown that the antioxidant effect of lithium varies, depending on the specific brain region and treatment regimen [47-49].

The present study found a negative correlation between locomotor activity and GSH levels in the PFC in sleep-deprived animals. When considering all of the animals together, a negative correlation was found between locomotor activity and GSH levels in the PFC. The CNS has a limited antioxidant capacity, and GSH is the main antioxidant in the brain [50]. Gawryluk et al. (2011) [51] showed that the post-mortem PFC in BD patients had lower levels of GSH, which can lead to higher susceptibility to neuronal oxidation, interfere with neuronal activity, and may contribute to the establishment of BD symptoms [52]. In the present study, PSD-induced hyperlocomotion was related to lower GSH levels in the PFC. Other studies have shown that the replenishment of GSH diminishes oxidative cellular damage and ameliorates the symptoms of BD [53, 54]. Our results also suggest that the hippocampus is less vulnerable to PSD-induced GSH depletion compared with the PFC and striatum.

Another parameter of oxidative stress that was analyzed in the present study was LPO. Lipids are the major components of cellular membranes and myelin sheaths that allow the conduction of neuronal signaling. Lipid peroxidation can dramatically compromise brain function [55]. High levels of oxidative damage to membrane phospholipids or the aggregation of oxidized proteins alters fluidity, which can induce cell death by apoptosis [56]. Under certain conditions, LPO causes structural disturbances, alterations in integrity, fluidity, and permeability, the functional loss of biomembranes, and the

generation of potentially toxic products [57]. Hydroperoxides are the major products of the free radical-mediated LPO of polyunsaturated fatty acids, and they are assumed to be pathogenic and contribute to the etiology of many diseases, including neurodegenerative diseases [58, 59]. Previous studies have reported that uncompensated oxidative stress increases LPO throughout the course of BD [60, 61]. In the present study, acute lithium administration and 10 and 40 mg/kg quercetin reversed the increases in LPO in the PFC, hippocampus, and striatum that were induced by PSD. These findings demonstrate the antioxidant and neuroprotective effects of both lithium and quercetin, suggesting antimanic-like effects of both drugs.

In the present study, a positive correlation was found between locomotor activity and LPO in the PFC and striatum in sleep-deprived mice. Our findings indicate that PSD-induced hyperlocomotion is correlated with higher LPO in the PFC and striatum. Brüning et al. (2012) [62] also reported a positive correlation between hyperlocomotion and indices of LPO.

Our research group has shown that the flavonoid myricitrin, which possesses antioxidant and PKC-inhibiting activities, is capable of reducing levels of oxidative stress and manic-like behavior [63, 64]. However, Valvassori et al. (2014) [65] reported that tamoxifen reduced levels of oxidative stress, although it does not possess antioxidant properties. This indicates that different mechanisms are involved in the lower levels of oxidative stress, which does not necessarily require antioxidant effects. Therefore, we can only hypothesize that the antioxidant effect of quercetin might be responsible for the reduction of the PSD-induced increase in LPO levels. Likewise, it is only possible to imply that the antioxidant effect of this flavonoid might contribute to its antimanic-like effect.

Our results indicate that quercetin decreases PSD-induced hyperlocomotion and displays an antimanic-like effect. Our results also indicated an antioxidant effect of quercetin, which may be related to its antimanic-like effect. Similar results were found for lithium, which is currently the

treatment of choice for BD. The antioxidant and PKC inhibitor quercetin may be an alternative therapeutic agent for the treatment of manic symptoms.

5. Conclusions

Overall, the present study showed that quercetin prevented PSD-induced hyperlocomotion in mice at doses that did not impair locomotor activity, suggesting an antimanic-like effect. This flavonoid also reversed the PSD-induced decrease in GSH levels in the PFC and striatum and PSD-induced increase in LPO in the PFC, hippocampus, and striatum. These behavioral and neurochemical effects appeared to be correlated, suggesting that quercetin may exert its actions by reducing oxidative stress.

Contributors

Roberto Andreatini and Inara MR Barcaro proposed the study. Luiz K.S. Kanazawa, Débora Dalla Vecchia, Etiéli Mara Wendler, and Palloma A.S. Hocayen conducted the behavioral tests. Luiz K.S. Kanazawa, Francislaine A.R. Lívero, Maria Carolina Stipp, and Alexandra Acco were responsible for evaluating oxidative stress. All of the authors contributed to the data analysis and interpretation, wrote the manuscript, and approved the final article.

Conflicts of interest

All of the authors declare that they have no conflicts of interest.

Acknowledgements

RA and AA are recipients of a research fellowship from CNPq. LKSK, DDV, EMW, PASH, FARL, MCS, and IMRB are recipients of a graduate fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

[1] Abrial, E.; Bétourné, A.; Etiévant, A.; Lucas, G.; Scarna, H.; Lambás-Señas, L.; Haddjeri, N. Protein kinase C inhibition rescues manic-like behaviors and hippocampal cell proliferation deficits in the sleep deprivation model of mania. **International Journal of Neuropsychopharmacology**. 18(2); pii: pyu031, 2015.

[2] Price, A. L.; Marzani-Nissen, G. R. Bipolar disorders: a review. **American Family Physician**. 85(5); 483-493, 2012.

[3] Merikangas, K. R.; Akiskal, H. S.; Angst, J.; Greenberg, P. E.; Hirschfeld, R. M.; Petukhova, M.; Kessler, R. C. Lifetime and 12-month prevalence of bipolar spectrum disorder in the National Comorbidity Survey replication. **Archives of General Psychiatry**. 64(5); 543-552, 2007 [erratum: 64(9); 1039].

[4] Michalak, E.; Livingston, J. D.; Hole, R.; Suto, M.; Hale, S.; Haddock, C. "It's something that I manage but it is not who I am": reflections on internalized stigma in individuals with bipolar disorder. **Chronic Illness**. 7 (3); 209-224, 2011.

[5] Chiu, C. T.; Wang, Z.; Hunsberger, J. G.; Chuang, D. M. Therapeutic potential of mood stabilizers lithium and valproic acid: beyond bipolar disorder. **Pharmacological Reviews**. 65(1); 105-142, 2013.

[6] Miklowitz, D. J.; Johnson, S. L. The psychopathology and treatment of bipolar disorder. **Annual Reviews in Clinical Psychology**. 2(1); 199-235, 2006.

[7] Müller-Oerlinghausen, B.; Berghöfer, A.; Bauer, M. Bipolar disorder. **Lancet**. 359(9302); 241-247, 2002.

[8] Kemp, D. E.; Sylvia, L. G.; Calabrese, J. R.; Nierenberg, A. A.; Thase, M. E.; Reilly-Harrington, N. A.; Ostacher, M. J.; Leon, A. C.; Ketter, T. A.; Friedman, E. S.; Bowden, C. L.; Pencina, M.; Iosifescu, D. V. General medical burden in bipolar disorder: findings from the LiTMUS comparative effectiveness trial. **Acta Psychiatrica Scandinavica**. 129(1); 24-34, 2014.

[9] Sajatovic, M.; Davies, M.; Hrouda, D. R. Enhancement of treatment adherence among patients with bipolar disorder. **Psychiatric Services**. 55(3); 264-269, 2004.

[10] Perlis, R. H.; Ostacher, M. J.; Patel, J. K.; Marangell, L. B.; Zhang, H.; Wisniewski, S. R.; Ketter, T. A.; Miklowitz, D. J.; Otto, M. W.; Gyulai, L.;

Reilly-Harrington, N. A.; Nierenberg, A. A.; Sachs, G. S.; Thase, M. E. Predictors of recurrence in bipolar disorder: primary outcomes from the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). **American Journal of Psychiatry**. 163(2); 217-224, 2006.

[11] Judd, L. L.; Akiskal, H. S.; Schettler, P. J.; Endicott, J.; Leon, A. C.; Solomon, D. A.; Coryell, W.; Maser, J. D.; Keller, M. B. Psychosocial disability in the course of bipolar I and II disorders: a prospective, comparative, longitudinal study. **Archives of General Psychiatry**. 62(12); 1322-1330, 2005.

[12] Lahera, G.; Freund, N.; Saiz-Ruiz, J. Salience and dysregulation of the dopaminergic system. **Revista de Psiquiatria y Salud Mental**. 6(1); 45-51, 2013.

[13] Hashimoto, K.; Sawa, A.; Iyo, M. Increased levels of glutamate in brains from patients with mood disorders. **Journal of Psychiatric Neuroscience and Therapeutics**. 62(11); 1310-1316, 2007.

[14] Manji, H. K.; Lenox, R. H. Signaling: cellular insights into the pathophysiology of bipolar disorder. **Biological Psychiatry**. 48(6); 518-530, 2000.

[15] Andreazza, A. C.; Kauer-Sant'anna, M.; Frey, B. N.; Bond, D. J.; Kapczkinski, F.; Young, T.; Yatham, L. N. Oxidative stress markers in bipolar disorder: a meta-analysis. **Journal of Affective Disorders**. 111(2-3); 135-144, 2008.

[16] Berk, M.; Kapczinski, F.; Andreazza, A. C.; Dean, O. M.; Giorlando, F.; Maes, M.; Yücel, M.; Gama, C. S.; Dodd, S.; Dean, B.; Magalhães, P. V. S.; Amminger, P.; McGorry, P.; Malhi, G. S. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. **Neuroscience and Biobehavioral Reviews**. 35(3); 804-817, 2011.

[17] Shao, L.; Young, L. T.; Wang, J. F. Chronic treatment with mood stabilizers lithium and valproate prevents excitotoxicity by inhibiting oxidative stress in rat cerebral cortical cells. **Biological Psychiatry**. 58(11); 879-884, 2005.

[18] Souza, L. C.; Wilhelm, E. A.; Bortolatto, C. F.; Nogueira, C. W.; Boeira, S. P.; Jesse, C. R. The protective effect of melatonin against brain oxidative stress and hyperlocomotion in a rat model of mania induced by ouabain. **Behavioural Brain Research**. 271; 316-324, 2014.

[19] Wang, H. Y.; Friedman, E. Lithium inhibition of protein kinase C activation-induced serotonin release. **Psychopharmacology**. 99(2); 213-218, 1989.

[20] Wang, H. Y.; Friedman, E. Enhanced protein kinase C activity and translocation in bipolar affective disorder brains. **Biological Psychiatry**. 40(7); 568-575, 1996.

[21] Gamet-Payrastre, L.; Manenti, S.; Gratacap, M. P.; Tulliez, J.; Chap, H.; Payrastre, B. Flavonoids and the inhibition of PKC and PI 3-kinase. **General Pharmacology**. 32(3); 279-286, 1999.

[22] Dajas, F. Abin-Carriquiry, J. A.; Arredondo, F.; Blasina, F.; Echeverry, C.; Martínez, M.; Rivera, F.; Vaamonde, L. Quercetin in brain diseases: potential and limits. **Neurochemistry International**. 89; 140-148, 2015.

[23] Lakhanpal, P.; Rai, D. K. Quercetin: a versatile flavonoid. **Internet Journal of Medical Update**. 2(2); 22-37, 2007.

[24] De Groot, H. Reactive oxygen species in tissue injury. **Hepatogastroenterology**. 41(4); 328-332, 1994.

[25] Grace, P. A. Ischemia-reperfusion injury. **British Journal of Surgery**. 81(5); 637-647, 1994.

[26] Rinwa, P.; Kumar, A. Quercetin suppress microglial neuroinflammatory response and induce antidepressent[sic]-like effect in olfactory bulbectomized rats. **Neuroscience**. 255; 86-98, 2013.

[27] Streck, E. L.; Scaini, G.; Jeremias, G. C.; Rezin, G. T.; Gonçalves, C. L.; Ferreira, G. K.; Réus, G. Z.; Resende, W. R.; Valvassori, S. S.; Kapczinski, F.; Andersen, M. L.; Quevedo, J. Effects of mood stabilizers on brain energy metabolism in mice submitted to an animal model of mania induced by paradoxical sleep deprivation. **Neurochemical Research**. 40(6); 1144-1152, 2015.

[28] Gessa, G. L.; Pani, L.; Fadda, P.; Fratta, W. Sleep deprivation in the rat: an animal model of mania. **European Neuropsychopharmacology**. 5(Suppl.); 89-93, 1995.

[29] Tufik, S.; Lindsey, C. J.; Carlini, E. A. Does REM sleep deprivation induce a supersensitivity of dopaminergic receptors in the rat brain? **Pharmacology**. 16(2); 98 – 105, 1978.

[30] Ghosh, P. K.; Hrdina, P. D.; Ling, G. M. Effects of REMS deprivation on striatal dopamine and acetylcholine in rats. **Pharmacology, Biochemistry and Behavior**. 4(4); 401-405, 1976.

[31] Silva, R. H.; Abílio, V. C.; Takatsu, A. L.; Kameda, S. R.; Grassl, C.; Chehin, A. B.; Medrano, W. A.; Calzavara, M. B.; Registro, S.; Andersen, M. L.; Machado, R. B.; Carvalho, R. C.; Ribeiro, R. A.; Tufik, S.; Frussa-

Filho, R. Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. **Neuropharmacology**. 46(6); 895-903, 2004.

[32] Bhutada, P.; Mundhada, Y.; Bansod, K.; Ubgade, A.; Quazi, M.; Umathe, S.; Mundhada, D. Reversal by quercetin of corticotrophin releasing factor induced anxiety- and depression-like effect in mice. **Progress in Neuropsychopharmacology amd Biological Psychiatry**. 34(6); 955-960, 2010.

[33] Sabioni, P.; Baretta, I. P.; Ninomiya, E. M.; Gustafson, L.; Rodrigues, A. L.; Andreatini, R. The antimanic-like effect of tamoxifen: behavioral comparison with other PKC-inhibiting and antiestrogenic drugs. **Progress in Neuropsychopharmacology and Biological Psychiatry**. 32(8); 1927-1931, 2008.

[34] Pereira, M.; Martynhak, B. J.; Baretta, I. P.; Correia, D.; Siba, I. P.; Andreatini, R. Antimanic-like effect of tamoxifen is not reproduced by acute or chronic administration of medroxyprogesterone or clomiphene**.** **Neuroscience Letters**. 500(2); 95-98, 2011.

[35] Mishra K.; Ojha, H.; Chaudhury, N. K. Estimation of antiradical properties of antioxidants using DPPH assay: a critical review and results. **Food Chemistry**. 130(4); 1036-1043, 2012.

[36] Blois, M. S. Antioxidant determinations by use of a stable free radical. **Nature**. 181; 1199-1200, 1958.

[37] Chen, F. A.; Wu, A. B.; Chen, C. Y. The influence of treatments on the free radical scavenging activity of burdock and variations of its activity. **Food Chemistry**. 86(4); 479-484, 2004.

[38] Sedlak, J.; Lindsay, R. H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. **Analytical Biochemistry**. 25(1); 192-205, 1968.

[39] Jiang, Z. Y.; Hunt, J. V.; Wolff, S. P. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. **Analytical Biochemistry**. 202(2); 384-389, 1992.

[40] Einat, H. Modelling facets of mania: new directions related to the notion of endophenotypes. **Journal of Psychopharmacology**. 20(5); 714-722, 2006.

[41] Harvey, A. G.; Talbot, L. S.; Gershon, A. Sleep disturbance in bipolar disorder across the lifespan. **Clinical Psychology Review**. 16(2); 256-277, 2009.

[42] Rumble, M. E.; White, K. H.; Benca, R. M. Sleep disturbances in mood disorders. **Psychiatric Clinics of North America**. 38(4); 743-759, 2015.

[43] Colombo, C.; Benedetti, F.; Barbini, B.; Campori, E.; Smeraldi, E. Rate of switch from depression into mania after therapeutic sleep deprivation in bipolar depression. **Psychiatry Research**. 86(3); 267-270, 1999.

[44] Minassian, A.; Young, J. W.; Cope, Z. A.; Henry, B. L.; Geyer, M. A.; Perry, W. Amphetamine increases activity but not exploration in humans and mice. **Psychopharmacology**. 233(2); 225-233, 2016.

[45] Berk, M.; Dodd, S.; Kauer-Sant'anna, M.; Malhi, G. S.; Bourin, M.; Kapczinski, F.; Norman, T. Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. **Acta Psychiatrica Scandinavica Supplementum**. 434; 41-49, 2007.

[46] Rees, J. N.; Florang, V. R.; Anderson, D. G.; Doorn, J. A. Lipid peroxidation products inhibit dopamine catabolism yielding aberrant levels of a reactive intermediate. **Chemical Research In Toxicology**. 20(10); 1536-1542, 2007.

[47] Frey, B. N.; Valvassori, S. S.; Réus, G. Z.; Martins, M. R.; Petronilho, F. C.; Bardini, K.; Dal-Pizzol, F.; Kapczinski, F.; Quevedo, J. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. **Reviews on Psychiatric Neuroscience**. 31(5); 326-332, 2006.

[48] Bhalla, P.; Dhawan, D. K. Protective role of lithium in ameliorating the aluminium-induced oxidative stress and histological changes in rat brain. **Cellular and Molecular Neurobiology**. 29(4); 513-521, 2009.

[49] Jornada, L. K.; Valvassori, S. S.; Steckert, A. V.; Moretti, M.; Mina, F.; Ferreira, C. L.; Arent, C. O.; Dal-Pizzol, F.; Quevedo, J. Lithium and valproate modulate antioxidant enzymes and prevent ouabain-induced oxidative damage in an animal model of mania. **Journal of Psychiatric Research**. 45(2); 162-168, 2011.

[50] Roda, A.; Chendo, I.; Kunz, M. Biomarkers and staging of bipolar disorder: a systematic review. **Trends in Psychiatry and Psychotherapy**. 37(1); 3-11, 2015.

[51] Gawryluk, J. W.; Wang, J. F.; Andreazza, A. C.; Shao, L.; Young, L. T. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders.

International Journal of Neuropsychopharmacology. 14(1); 123-130, 2011.

[52] Dringen, R. Metabolism and functions of glutathione in brain. **Progress in Neurobiology**. 62(6); 649-671, 2000.

[53] Dean, O. M.; van den Buuse, M.; Bush, A. I.; Copolov, D. L.; Ng, F.; Dodd, S.; Berk, M. A role for glutathione in the pathophysiology of bipolar disorder and schizophrenia? Animal models and relevance to clinical practice. **Current Medicinal Chemistry**. 16(23); 2965-2976, 2009.

[54] Magalhães, P. V.; Dean, O. M.; Bush, A. I.; Copolov, D. L.; Malhi, G. S.; Kohlmann, K.; Jeavons, S.; Schapkaitz, I.; Anderson-Hunt, M.; Berk, M. N-acetylcysteine for major depressive episodes in bipolar disorder. **Revista Brasileira de Psiquiatria**. 33(4); 374-378, 2011.

[55] Joshi, Y. B.; Praticò, D. Lipid peroxidation in psychiatric illness: overview of clinical evidence. **Oxidative Medicine and Cellular Longevity**. 2014; 828702, 2014.

[56] Mahadik, S. P.; Evans, D.; Lal, H. Oxidative stress and role of antioxidant and omega-3 essential fatty acid supplementation in schizophrenia. **Progress in Neuropsychopharmacology and Biological Psychiatry**. 25(3); 463-493, 2001.

[57] Greenberg, M. E.; Li, X. M.; Gugiu, B. G.; Gu, X.; Qin, J.; Salomon, R. G.; Hazen, S. L. The lipid Whisker model of the structure of oxidized cell membranes. **Journal of Biological Chemistry**. 283(4); 2385-2396, 2008.

[58] Niki, E. Lipid peroxidation: physiological levels and dual biological effects. **Free Radical Biology and Medicine**. 47(5); 469-484, 2009.

[59] Sultana, R.; Perluigi, M.; Butterfield, D. A. Protein oxidation and lipid peroxidation in brain of subjects with Alzheimer's disease: insights into mechanism of neurodegeneration from redox proteomics. **Antioxidants and Redox Signaling**. 8(11-12); 2021-2037, 2006.

[60] Andreazza, A. C.; Cassini, C.; Rosa, A. R.; Leite, M. C.; de Almeida, L. M.; Nardin, P.; Cunha, A. B.; Ceresér, K. M.; Santin, A.; Gottfried, C.; Salvador, M.; Kapczinski, F.; Gonçalves, C. A. Serum 100B and antioxidant enzymes in bipolar patients. **Journal of Pyschiatric Research**. 41(6); 523-529, 2007.

[61] Machado-Vieira, R.; Andreazza, A. C.; Viale, C. I.; Zanatto, V.; Ceresér, V., Jr.; da Silva Vargas, R.; Kapczinski, F.; Portela, L. V.; Souza, D. O.; Salvador, M.; Gentil, V. Oxidative stress parameters in unmedicated and

treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects. **Neuroscience Letters**. 421(1); 33-36, 2007.

[62] Brüning, C. A.; Prigol, M.; Luchese, C.; Pinton, S.; Nogueira, C. W. Diphenyl diselenide ameliorates behavioral and oxidative parameters in an animal model of mania induced by ouabain. **Progress in Neuropsychopharmacology and Biological Psychiatry**. 38(2); 168-174, 2012.

[63] Pereira, M.; Andreatini, R.; Schwarting, R. K.; Brenes, J. C. Amphetamine-induced appetitive 50-kHz calls in rats: a marker of affect in mania? **Psychopharmacology**. 231(13); 2567-2577, 2014.

[64] Andreatini, R.; Pereira, M.; Siba, I. P.; Pizzolatti, M. G.; Santos, A. R. S.; Ruani, A. P. Myricitrin, a PKC inhibitor, exerts antimanic-like effects in animal models. **European Neuropsychopharmacology**. 23(Suppl. 2); S357-S358, 2013.

[65] Valvassori, S. S.; Bavaresco, D. V.; Budni, J.; Bobsin, T. S.; Gonçalves, C. L.; de Freitas, K. V.; Streck, E. L.; Quevedo, J. Effects of tamoxifen on tricarboxylic acid cycle enzymes in the brain of rats submitted to an animal model of mania induced by amphetamine. **Psychiatry Research**. 215(2); 483-487, 2014.

4. ARTIGO 2

Effects of acute and chronic administration of quercetin on hyperlocomotion and oxidative stress induced by methylphenidate

Luiz K. S. Kanazawa,[a] Débora D. Vecchia,[a] Etiéli M. Wendler,[a] Palloma de A. S. Hocayen,[a] Paulo S. B. Júnior,[a] Manuela L. de Mélo,[a] Francislaine A. dos Reis Lívero,[b] Maria Carolina Stipp,[b] Inara M. R. Barcaro,[a] Alexandra Acco,[b] Roberto Andreatini[a,] *

[a] Laboratory of Physiology and Pharmacology of the Central Nervous System, Department of Pharmacology, Federal University of Paraná, Centro Politécnico, 81540-990, Curitiba, PR, Brazil

[b] Laboratory of Pharmacology and Metabolism, Department of Pharmacology, Federal University of Paraná, Centro Politécnico, 81540-990, Curitiba, PR, Brazil

*Corresponding author

ABSTRACT

Despite the availability of many pharmacological agents for treating mania in bipolar disorder (BD), their management remains a challenge for various reasons. Therefore, it is important to instigate the research for new drugs with antimanic properties. In this context, we hypothesized that the flavonoid quercetin might exert antimanic-like effects due to its antioxidant and PKC-inhibiting activities, which resemble lithium, the first-line treatment for mania in BD. In the present study, we investigated the effect of acute and chronic treatment with quercetin (2.5, 5, 10 and 40 mg/kg i.p.) of male Swiss mice submitted to the methylphenidate-induced hyperlocomotion animal model of mania, using lithium as positive control. In addition, we analyzed the effects of these drugs in methylphenidate-induced brain oxidative stress by measuring reduced glutathione (GSH) and lipid peroxidation (LPO) levels in the prefrontal cortex (PFC), hippocampus and striatum. We observed that chronic treatment with 10 and 40 mg/kg quercetin blocked methylphenidate-induced hyperlocomotion, showing an antimanic-like effect of quercetin. In addition, chronic treatment with lithium or quercetin blocked methylphenidate-induced increase in LPO levels in the striatum. These results suggest that quercetin displays antimanic-like and antioxidant effects, which makes this flavonoid a relevant agent for the research of new antimanic drugs.

Keywords: Bipolar disorder, hyperlocomotion, mania, oxidative stress, methylphenidate, protein kinase C, quercetin.

Abbreviations: BD, bipolar disorder; CMC, carboxymethylcellulose; DTNB, 5,5'-dithiobis-(2-nitrobenzoic acid); GSH, reduced glutathione; LPO, lipid peroxidation; PFC, prefrontal cortex; PKC, protein kinase C; RNS, reactive nitrogen species; ROS, reactive oxygen species.

1. Introduction

Bipolar patients with a manic episode can be treated with mood stabilizers such as lithium, antipsychotics and anticonvulsants (Hoertel et al., 2013). Despite the availability of many pharmacological agents for treating mania in bipolar disorder (BD), their management in clinical settings remains a challenge (Nivoli et al., 2012). It is challenging, not only due to the potentially severe course of the disorder, where the patient may present a broad range of symptoms and an unpredictable cycling mood state, but also due to its complex and alternating clinical picture and to the low responsitivity of patients to treatment (Hoertel et al., 2013, Schuepbach, 2015).

The pathophysiology of BD involves a large number of pathways, which makes it difficult to discover and develop a drug to effficaciously treat such a heterogeneous disease. Therefore, the use of several therapeutic agents that target multiple signaling nodes and that present less side effects is required to improve treatment for BD (Hoertel et al., 2013). Studies show that, among other factors, the pathophysiology of mania in BD involves increased levels of oxidative stress (Andreazza et al., 2008; Berk et al., 2011) and increased activity of protein kinase C (PKC) (Manji and Lenox, 2000). In this sense, we hypothesize that the flavonoid quercetin might have an antimanic-like effect, basically due to its antioxidant properties (Dajas et al., 2015) and inhibitory activity over the enzyme PKC (Gamet-Payrastre et al., 1999), similarly to lithium, the first-line treatment for mania in BD (Frey et al., 2006; Wang and Friedman, 1989).

One of the animal models employed to induce manic-like behaviors is the administration of psychostimulants, such as methylphenidate. Pharmacological induction of manic-like behaviors, in this case hyperlocomotion, is easy to generate, easy to test and shows reliability and validity. Locomotor activity is known to be increased in manic patients (Young et al., 2007). Mines et al. (2013) showed that methylphenidate administration increases locomotor activity of mice.

Quercetin displays antioxidant and PKC inhibiting activity, like lithium, the gold standard treatment for mania. Therefore, the objective of this study was to evaluate the effects of acute and chronic administration of quercetin on methylphenidate-induced hyperlocomotion and brain oxidative stress in mice.

2. Material and methods

2.1 *Animals*

The experiments were conducted using male Swiss mice weighing between 30 and 40 g and housed at 22ºC ± 2ºC under a 12 h/12 h light/dark cycle (lights on at 7:00 AM). The animals were kept in polypropylene cages (41 cm x 34 cm x 16 cm) with food and water available at all times. All of the experiments were approved by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA/BIO-UFPR, protocol no. 733).

2.2 *Drugs*

The mice were treated with saline (0.9% NaCl; 10 ml/kg, i.p.), lithium carbonate (Eurofarma, Brazil; positive control; 100 mg/kg, i.p., dissolved in saline, with the pH adjusted to 7.4 with hydrochloric acid), diazepam (Cristália, Brazil; 5 mg/kg i.p., dissolved in distilled water) or quercetin (Sigma, St. Louis, MO, USA; 2.5, 5, 10 or 40 mg/kg, i.p., suspended in 0.5% carboxymethylcellulose [CMC]). Drugs were administered in a volume of 10 ml/kg of body weight. The doses were based on data from the literature (Bhutada et al., 2010) and previous studies by our research group (Sabioni et al., 2008; Pereira et al., 2011). Chronic treatment with lithium, diazepam and quercetin lasted for 21 days, once a day.

2.3 *Methylphenidate-induced hyperlocomotion protocol*

For the acute treatment protocol, the animals were pretreated with lithium, diazepam or quercetin 15 minutes before the administration of either vehicle or methylphenidate (Fig. 1). For the chronic treatment protocol, the animals were pretreated with lithium, diazepam or quercetin 21 days before vehicle of methylphenidate administration, which were made 15 minutes after the last treatment with lithium, diazepam or quercetin (Fig. 2).

Twenty minutes after vehicle or methylphenidate administration, the animals were placed individually for 20 minutes in the automated activity box, which is a wooden box (40 x 20 x 26 cm) with a wire mesh floor which has three photoelectric sensors (10 cm apart from one another). The number of crossings was registered by the photoelectric sensors.

In the end, the animals were taken from the box and put back to their home cage. The number of crossings is considered to be an index of locomotor activity, and an increase after methylphenidate administration indicates a stimulant effect. A blockade of the stimulant effect of methylphenidate, at a dose that did not decrease locomotor activity, indicates an antimanic-like effect (Gould et al., 2001; Sabioni et al., 2008).

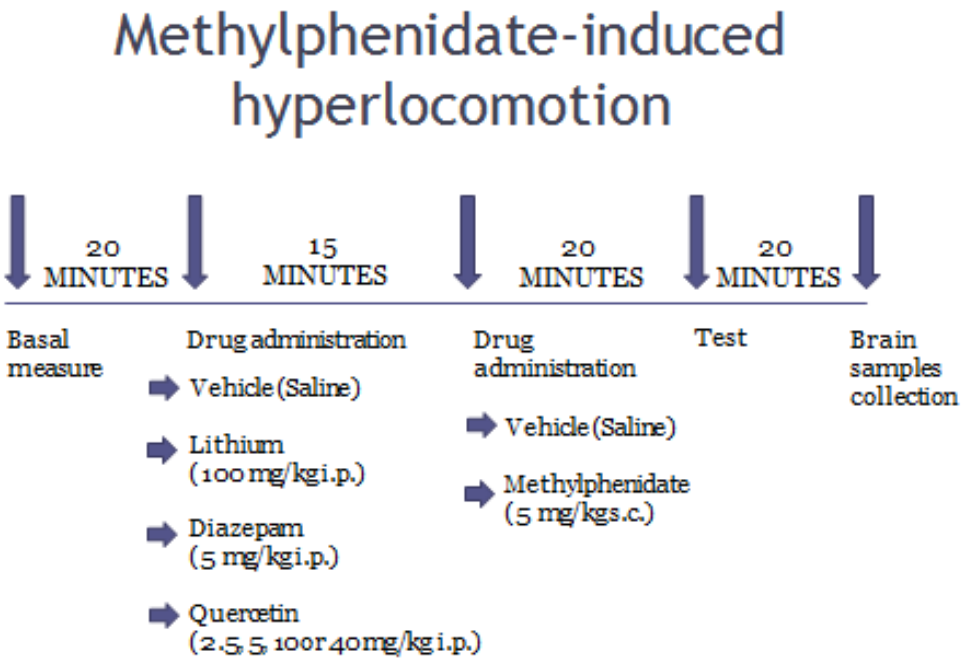


Fig. 1 – Timeline for the induction of hyperlocomotion with methylphenidate in mice in the acute treatment protocol.

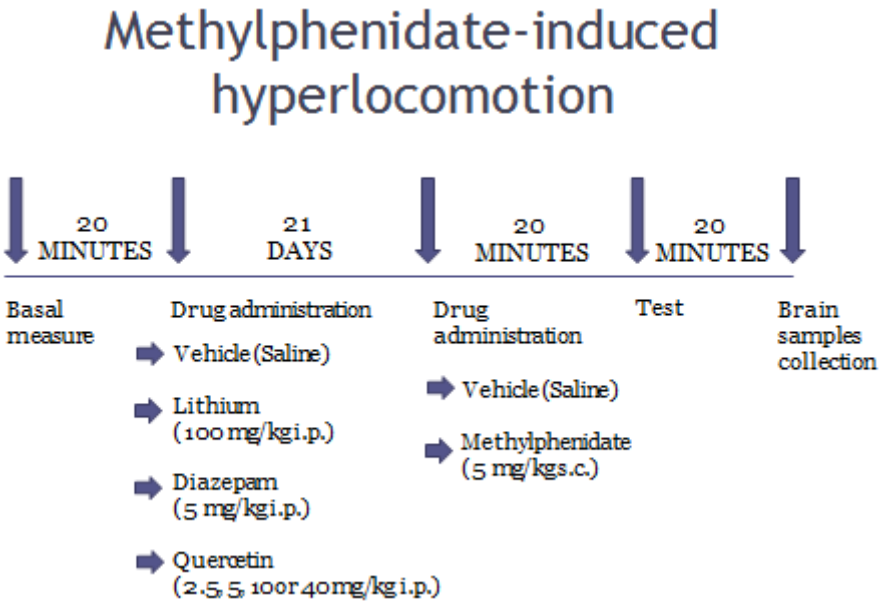


Fig. 2 – Timeline for the induction of hyperlocomotion with methylphenidate in mice in the chronic (21 days) treatment protocol.

2.5 *Evaluation of oxidative stress parameters in the mice brains*

2.5.1 *Brain samples*

The mice were decapitated immediately after being exposed to the automated activity box. The prefrontal cortex (PFC), hippocampus, and striatum were dissected, frozen in liquid nitrogen, and then stored at -80ºC until further analysis.

The samples were homogenized in potassium phosphate buffer (0.1 M, pH 6.5) in a 1:10 dilution. One part of the homogenate was used to evaluate GSH levels, and the other part was centrifuged at 9000 $\times$ *g* in a micro-high-speed refrigerated centrifuge (VS-15000 CFNII, Vision Scientific, Daejeon, South Korea) for 20 min. The supernatant was used to evaluate LPO levels.

2.6 *Evaluation of reduced glutathione (GSH) levels*

To evaluate GSH levels, 100 μl of the homogenate was mixed with 80 μl of 12.5% trichloroacetic acid and centrifuged at 6000 rotations per minute (rpm) for 15 min at 4ºC. Then, 20 μl of the supernatant was mixed with 280 μl of Tris buffer (0.4 M, pH 8.9) and 5 μl of DTNB (5,5'-dithiobis-[2-nitrobenzoic acid] 0.01 M) following the protocol that was originally described by Sedlak and Lindsay (1968), with minor modifications. Absorbance was read at 415 nm using a multi-mode microplate reader (BioTek Synergy HT, BioTek Instruments, Winooski, VT, USA). The individual values were then interpolated in a standard curve of GSH (0.375-3 μg) to verify the linearity of the reaction (r^2 must be > 0.99), and the values were divided by a correction factor. The results are expressed as μg/g of tissue, which represents the quantity of GSH (μg) in the tissue (g).

2.7 *Evaluation of lipid peroxidation (LPO) levels*

The LPO levels were measured following the method described by Jiang et al. (1992), with minor modifications. Initially, 100 μl of the supernatant was suspended in 100 μl of methanol, vortexed, and then centrifuged at 5000 rpm for 5 min at 4ºC. After, 100 μl of the supernatant was added to 900 μl of FOX2 reagent (4 mM BHT, 250 μM $FeSO_4$, 250 mM H_2SO_4, and 100 mM xylenol orange). The samples were vortexed and incubated in the dark for 30 min at room temperature. Absorbance was read at 560 nm using a multi-mode microplate reader (BioTek Synergy HT, BioTek Instruments, Winooski, VT, USA). The results are expressed as mmol of hydroperoxides/mg of tissue.

2.9 *Statistical analysis*

For all of the experiments, two-way analysis of variance (ANOVA) was used. If significant main effects or interactions were found in the ANOVA, the Newman-Keuls *post hoc* test was used. The data are expressed as means ± S.E.M. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1 Behavioral test

3.1.1 Effect of acute administration of quercetin on methylphenidate-induced hyperlocomotion

There was a significant main effect of methylphenidate ($F_{1,182}$= 46.272, $p < 0.0001$) and treatment ($F_{6,182}$ = 4.760, $p < 0.001$) and methylphenidate by treatment interaction ($F_{6,182}$ = 1.121, $p = 0.35$). Lithium and diazepam were both capable of blocking the effect of methylphenidate ($p < 0.05$). However,

diazepam alone reduced locomotor activity ($p < 0.05$ compared to veh + veh). No effect was observed at any dose of quercetin (Fig. 3).

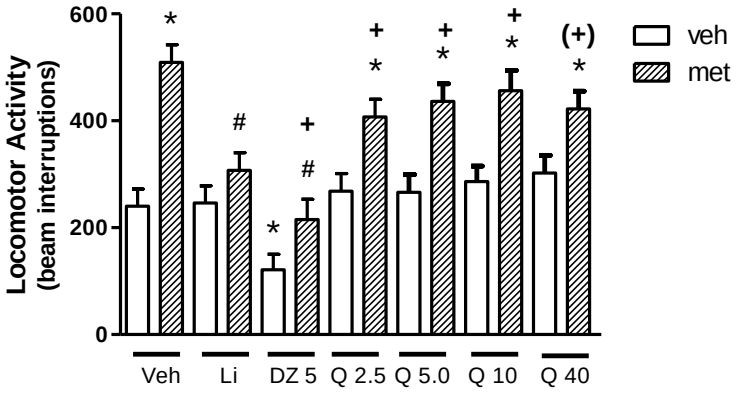


Fig 3. Effect of acute quercetin (Q; 2.5-40 mg/kg, i.p.), lithium (Li; 100 mg/kg, i.p.) and diazepam (DZ; 5 mg/kg, i.p.) administration on methylphenidate-induced hyperlocomotion. Veh: vehicle; met: methylphenidate. The data are expressed as the mean ± SEM number of beam breaks over 20 min; n = 14 mice/group. *$p < 0.05$ compared with veh + veh; $^{\#}p < 0.05$ compared to veh + met; $^{(\#)}$ $0.05 < p < 0.10$ compared to veh + met; $^{+}p < 0.05$ compared to same drug + veh; $^{(+)}$ $0.05 < p < 0.10$ compared to same drug + veh.

3.1.2 Effect of chronic treatment of quercetin on methylphenidate-induced hyperlocomotion

There was a significant main effect of methylphenidate ($F_{1,42} = 131.9$, $p < 0.001$), treatment ($F_{6,42} = 14.90$, $p < 0.001$) and interaction ($F_{6,42} = 9.96$, $p < 0.001$). Methylphenidate increased locomotor activity compared to control (veh + veh ; $p < 0.01$; Fig. 4). Treatment with lithium, diazepam, 10 and 40 mg/kg quercetin were able to block methylphenidate-induced hyperlocomotion ($p < 0.05$). Treatment with 2.5 and 5 mg/kg quercetin attenuated methylphenidate-induced hyperlocomotion ($p < 0.05$). No effect of lithium or quercetin were seen in spontaneous locomotor activity. Treatment with diazepam alone significantly reduced locomotion ($p < 0.001$).

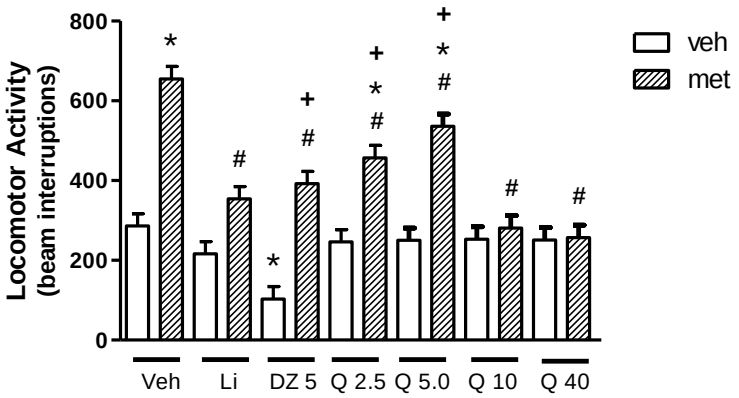


Fig 4. Effect of chronic quercetin (Q; 2.5-40 mg/kg, i.p.), lithium (Li; 100 mg/kg, i.p.) and diazepam (DZ; 5 mg/kg, i.p.) administration on methylphenidate-induced hyperlocomotion. Veh: vehicle; met: methylphenidate. The data are expressed as the mean ± SEM number of beam breaks over 20 min; n = 10-12 mice/group. $^{*}p < 0.05$ compared to veh + veh; $^{\#}p < 0.05$ compared to veh + met; $^{+}p < 0.05$ compared to same drug + veh.

3.2 Oxidative stress

3.2.1 Effect of acute administration of quercetin on GSH after methylphenidate-induced hyperlocomotion

Our results showed significant effect of methylphenidate ($F_{1,42}$= 60.421, $p < 0.001$), acute treatment ($F_{6,42}$= 5.028, $p < 0.001$) and the interaction between methylphenidate and quercetin administration ($F_{6,42}$= 8.742, $p < 0.001$) on GSH levels in the PFC. Methylphenidate administration increased GSH levels compared to the vehicle + vehicle group ($p < 0.001$). Lithium, diazepam, 5 and 10 mg/kg quercetin induced an increase in GSH levels ($p < 0.05$, compared to veh + veh). Treatment with 10 mg/kg quercetin reduced methylphenidate-induced increase in GSH levels ($p < 0.05$) (Fig. 5 A).

Two-way ANOVA of GSH levels in hippocampus showed significant effect of methylphenidate ($F_{1,40}$ = 16.831, $p < 0.001$) and treatment ($F_{6,40}$= 4.582, $p < 0.05$) administration. The p*ost hoc* comparisons indicated that methylphenidate did not alter GSH levels compared to the vehicle + vehicle

group. Treatment with 10 mg/kg quercetin alone and 5, 10 and 40 mg/kg quercetin + methylphenidate, increased GSH levels compared to the vehicle + vehicle group ($p < 0.05$) (Fig. 5 B).

Two-way ANOVA of GSH levels in the striatum showed significant main effect of methylphenidate administration ($F_{1,33}= 5.435$, $p < 0.05$) on GSH levels. However, the *post hoc* comparisons showed no difference among the groups. (Fig. 5 C).

3.2.2 Effect of chronic treatment of quercetin on GSH levels after methylphenidate-induced hyperlocomotion

We observed a signifcant effect of chronic treatment ($F_{6,42}= 18.651$, $p < 0.001$) on GSH levels in the PFC. The *post hoc* comparisons revealed that the veh + met group showed a tendency to increase GSH levels compared to the veh + veh group ($p = 0.058$), as well as treatment with 40 mg/kg quercetin ($p = 0.052$). Treatment with lithium and 10 mg/kg quercetin increased GSH levels compared to the veh + veh group ($p < 0.05$) and lithium was not capable of blocking the increase in GSH induced by methylphenidate ($p < 0.05$). Diazepam increased GSH levels compared to the veh + veh and to the veh + met groups ($p < 0.001$) (Fig. 6 A).

Two-way ANOVA of GSH levels in hippocampus showed significant effect of methylphenidate ($F_{1,40} = 42.781$, $p < 0.001$) and chronic treatment ($F_{6,40}= 4.776$, $p < 0.001$). The *post hoc* comparisons indicated that methylphenidate administration increased GSH levels ($p < 0.05$), when compared to the veh + veh group. (Fig. 6 B).

We observed a significant effect of methylphenidate ($F_{1,40} = 71.381$, $p < 0.001$) and chronic treatment ($F_{6,40}= 5.035$, $p < 0.001$) administration and also significant effect of the interaction between methylphenidate and chronic treatment ($F_{6,40}= 7.354$, $p < 0.001$) on GSH levels in the striatum. The *post hoc* test showed that the veh + met group had a tendency to show decreased levels of GSH compared to the veh + veh group ($p = 0.066$). Chronic treatment with

2.5 mg/kg quercetin increased the levels of GSH in the striatum when compared to the veh + veh group ($p < 0.05$). On the other hand, the 2.5 mg/kg quercetin + met group showed decreased levels of GSH when compared to the veh + veh group ($p < 0.05$). In the quercetin group, methylphenidate reduced striatal GSH levels compared to veh + same dose of quercetin, except for 5 mg/kg quercetin (all $p < 0.05$) (Fig. 6 C).

3.2.3 Effect of acute treatment of quercetin on LPO levels after methylphenidate-induced hyperlocomotion

Two-way ANOVA of LPO levels in the PFC revealed significant main effect of methylphenidate ($F_{1,41}= 7.027$, $p < 0.05$) and acute treatment ($F_{6,41}= 12.435$, $p < 0.001$). The *post hoc* test showed that no effect of methylphenidate was observed with methylphenidate administration (veh + veh compared to veh + met group). Moreover, 10 and 40 mg/kg ($p < 0.001$) quercetin increased LPO levels when co-administered with methylphenidate (Fig. 7 A).

Two-way ANOVA showed significant main effect of methylphenidate ($F_{1,40}= 18.093$, $p < 0.001$) and acute treatment ($F_{6,40}= 13.714$, $p < 0.001$) as well as the interaction between methylphenidate and acute treatment ($F_{6,40}= 3.927$, $p < 0.05$) on LPO levels in the hippocampus. Methylphenidate administration significantly increased LPO levels when compared to the veh + veh group ($p < 0.001$). Treatment with 5, 10 and 40 mg/kg quercetin alone increased LPO levels (5 and 10, $p < 0.001$; 40, $p < 0.05$) and were not capable of blocking methylphenidate-induced increase in LPO levels. Lithium and diazepam administration were able to block the effect of methylphenidate ($p < 0.05$) (Fig. 7 B).

We did not observe significant effect of methylphenidate or acute treatment in LPO levels in the striatum (both $p > 0.05$) (Fig. 7 C).

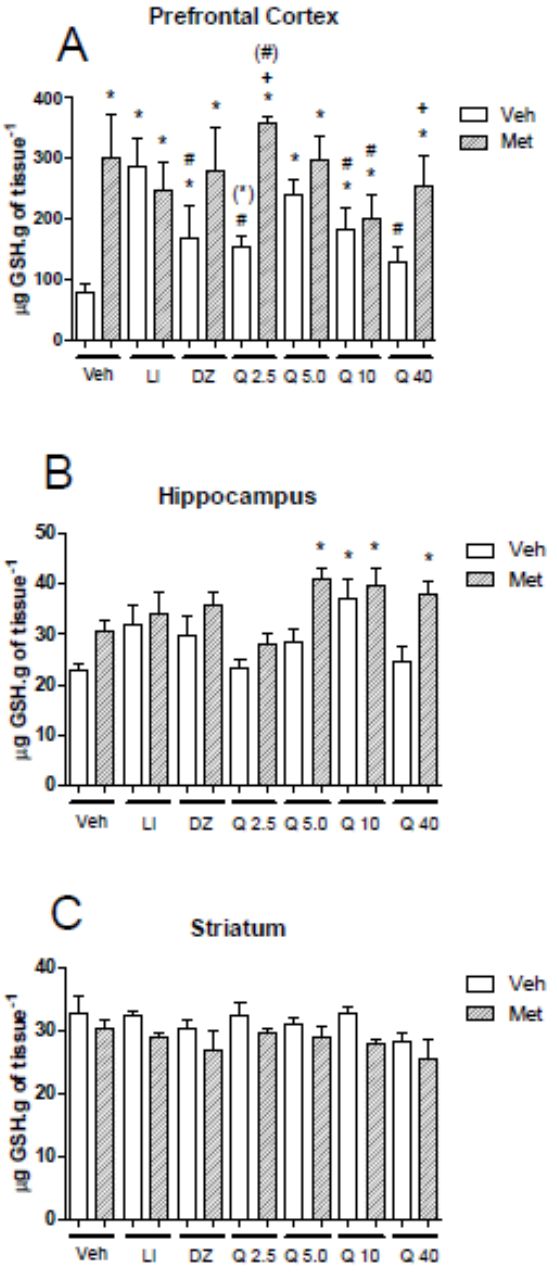


Fig. 5. Effect of acute quercetin (Q; 2.5-40 mg/kg, i.p.), lithium (Li; 100 mg/kg, i.p.) and diazepam (DZ; 5 mg/kg, i.p.) administration on GSH levels in the prefrontal cortex (A), hippocampus (B) and striatum (C) in mice that were or were not subjected to the methylphenidate-induced hyperlocomotion model. The data are expressed as mean ± SEM, n = 4 mice/group. *$p < 0.05$, compared to veh + veh; $^{\#}p < 0.05$ compared to veh + met; $^{+}p < 0.05$ compared to same drug + veh.

3.2.4 Effect of chronic treatment of quercetin on LPO levels after methylphenidate-induced hyperlocomotion

Two-way ANOVA of LPO levels in the PFC showed significant effect of methylphenidate ($F_{1,41}$= 41.499, $p < 0.001$) and chronic treatment ($F_{6,41}$= 3.961, $p < 0.01$). The *post hoc* test revealed that methylphenidate increased levels of LPO in the PFC when compared to the veh + veh group ($p < 0.001$), Treatment with 10 mg/kg quercetin was able to block methylphenidate-induced increase in LPO, while lithium, 2.5, 5 and 10 mg/kg quercetin attenuated it (Fig. 8 A).

There was also significant effect of methylphenidate ($F_{1,41}$= 16.893, $p < 0.001$), chronic treatment ($F_{6,41}$= 3.590, $p < 0.01$) and of the interaction between methylphenidate and chronic treatment ($F_{6,41}$= 4.779, $p < 0.001$) on LPO levels in the hippocampus. The *post hoc* comparisons showed that methylphenidate administration increased the levels of LPO in the hippocampus when compared to the veh + veh group ($p < 0.05$). Treatment with any dose of quercetin did not block methylphenidate-induced increase in LPO. Treatment with diazepam alone increased LPO levels ($p < 0.05$) (Fig. 8 B).

Two-way ANOVA of LPO levels in the striatum showed significant effect of methylphenidate ($F_{1,39}$= 33.884, $p < 0.001$), chronic treatment ($F_{6,39}$= 8.222, $p < 0.001$) and of the interaction between methylphenidate and chronic treatment administration ($F_{6,41}$= 3.248, $p < 0.05$). The *post hoc* test showed that methylphenidate administration increased LPO levels when compared to the vehicle + vehicle group ($p<0.001$). Treatment with lithium ($p<0.05$), 2.5 and 10 mg/kg quercetin ($p <0.05$) and 5.0 and 40 mg/kg quercetin ($p<0.001$) as well as with diazepam ($p<0.05$) were capable of decreasing LPO levels when compared to the vehicle + methylphenidate group (Fig. 8 C).

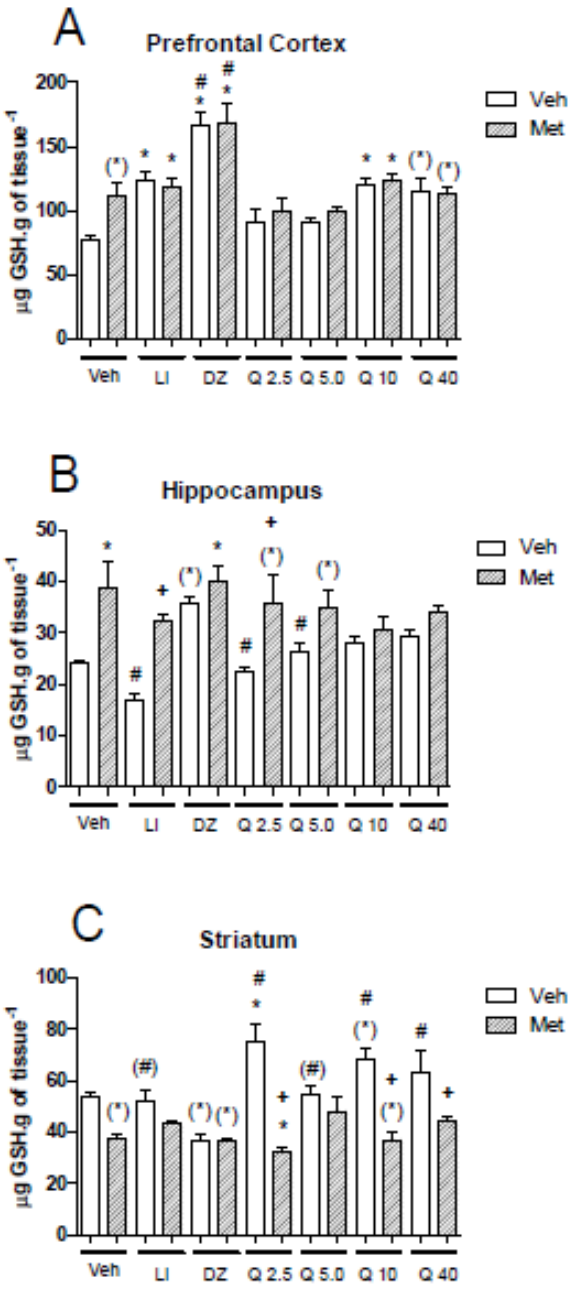


Fig. 6. Effect of chronic quercetin (Q; 2.5-40 mg/kg, i.p.), lithium (Li; 100 mg/kg, i.p.) and diazepam (DZ; 5 mg/kg, i.p.) administration on GSH levels in the prefrontal cortex (A), hippocampus (B) and striatum (C) in mice that were or were not subjected to the methylphenidate-induced hyperlocomotion model. The data are expressed as mean ± SEM, n = 4 mice/group. *$p < 0.05$ compared to veh + veh; $^{\#}p < 0.05$ compared to veh + met; (*) $0.05 < p < 0.10$, compared to veh + veh; ($^{\#}$) $0.05 < p < 0.10$, compared to veh + met; $^{+}p < 0.05$ compared to same drug + veh.

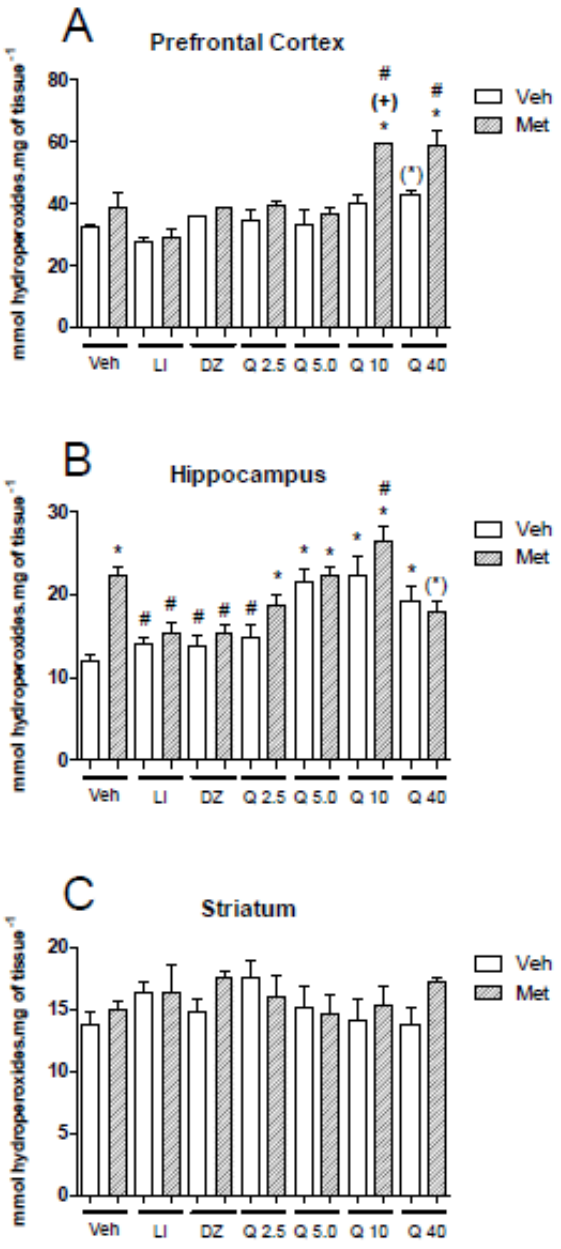


Fig. 7. Effect of acute quercetin (Q; 2.5-40 mg/kg, i.p.), lithium (Li; 100 mg/kg, i.p.) and diazepam (DZ; 5 mg/kg, i.p.) administration on LPO levels in the prefrontal cortex (A), hippocampus (B) and striatum (C) in mice that were or were not subjected to the methylphenidate-induced hyperlocomotion model. The data are expressed as mean ± SEM, n = 4 mice/group. *$p < 0.05$ compared to veh + veh; $^{\#}p < 0.05$ compared to veh + met. (*) $0.05 < p < 0.10$, compared to veh + veh; ($^{\#}$) $0.05 < p < 0.10$, compared to veh + met; $^{+}p < 0.05$ compared to same drug + veh; $^{(+)}$ $0.05 < p < 0.10$ compared to same drug + veh.

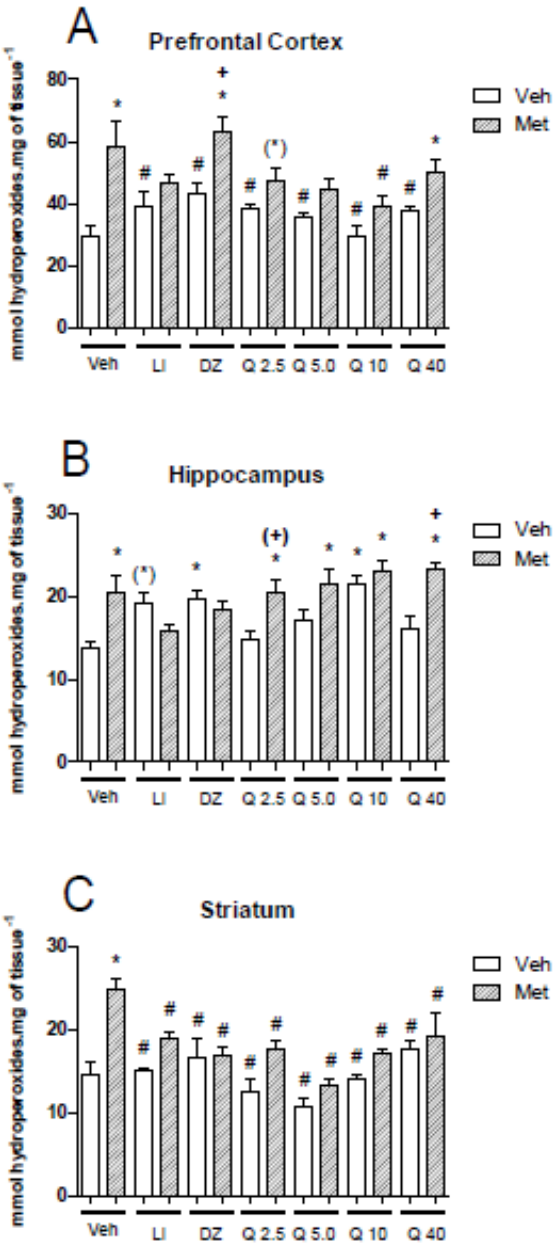


Fig. 8. Effect of chronic quercetin (Q; 2.5-40 mg/kg, i.p.), lithium (Li; 100 mg/kg, i.p.) and diazepam (DZ; 5 mg/kg, i.p.) administration on LPO levels in the prefrontal cortex (A), hippocampus (B) and striatum (C) in mice that were or were not subjected to the methylphenidate-induced hyperlocomotion model. The data are expressed as mean ± SEM, n = 4 mice/group. *$p < 0.05$ compared to veh + veh; $^{\#}p < 0.05$ compared to veh + met; $^{+}p < 0.05$ compared to same drug + veh; $^{(+)}$ $0.05 < p < 0.10$ compared to same drug + veh.

3.2.5 Correlation between methylphenidate-induced hyperlocomotion and GSH and LPO levels in the hippocampus, striatum and PFC.

Table 1 shows the correlation coefficients between methylphenidate-induced hyperlocomotion and oxidative stress indexes of mice belonging either to the acute or chronic treatment groups.

When considering all animals from the acute treatment procotol, there was a positive correlation between hyperlocomotion and LPO levels in the PFC (r = 0.48, p < 0.001) and hippocampus (r = 0.56, p < 0.001), as well as a positive correlation with GSH levels in the PFC (r = 0.38, p < 0.01). When considering only the animal that received methylphenidate, there was a positive correlation between hyperlocomotion and LPO levels in the hippocampus (r = 0.60, p < 0.001).

Regarding the animals of the chronic treatment protocol, when considering all animals, there was a positive correlation between hyperlocomotion and GSH levels in the hippocampus (r = 0.42, p < 0.001) and LPO levels in the PFC (r = 0.50, p < 0.001). Also, there was a negative correlation between hyperlocomotion and GSH levels in the striatum (r = -0.36, p < 0.01).

Table 1. Correlation coefficients (Pearson's r) between methylphenidate-induced hyperlocomotion and GSH and LPO levels in the hippocampus, striatum, and prefrontal cortex.

	Hippocampus		Striatum		Prefrontal cortex	
Locomotor activity	GSH	LPO	GSH	LPO	GSH	LPO
Acute treatment						
All mice (n = 47-56)	0.28*	0.56***	-0.17	-0.01	0.38**	0.48***
Methylphenidate (n = 22-26)	0.06	0.60***	0.07	-0.28	0.003	0.45*
Chronic treatment						
All mice (n = 53-56)	0.42***	0.09	-0.36**	0.32*	-0.22	0.50***
Methylphenidate (n = 26-28)	0.32	-0.21	-0.16	0.09	-0.32	0.35(*)

*p < 0.05, **p < 0.01, ***p < 0.001, (*) 0.01 < p < 0.05.

4. Discussion

Our results showed that chronic, but not acute, treatment with 10 mg/kg or 40 mg/kg quercetin was capable of blocking methylphenidate-induced hyperlocomotion. Both acute and chronic treatment with lithium blocked methylphenidate-induced hyperlocomotion, in line with its antimanic clinical effect. These results with lithium indicate the sensitivity and validity of the procedure. Thus, the effect of quercetin on methylphenidate-induced hyperlocomotion indicated an antimanic-like effect of quercetin. Diazepam was used as negative control. Acute and chronic treatment with diazepam blocked methylphenidate-induced hyperlocomotion. However, in both experiments, diazepam alone reduced locomotor activity, suggesting a sedative instead of an antimanic-like effect of diazepam (Young et al., 2011).

Psychostimulant-induced hyperlocomotion is the most frequently used animal model of mania (Einat, 2006). This pharmacological induction of manic-like behavior is reliable and shows face, construct and predictive validity (Einat, 2006; Machado-Vieira et al., 2004). Psychostimulants that are capable of increasing the levels of dopamine cause behavioral effects that resemble mania, such as hyperlocomotion (Jacobson and Silverstone, 1986). A reduction in dopaminergic activity ameliorates the symptoms of mania (Post et al., 1980). Lithium is capable of decreasing dopaminergic transmission (Dziedzicka-Wasylewska et al., 1996). Macêdo et al. (2013) showed that administration of the pro-drug lisdexamfetamine dimesylate, which is later metabolized to D-amphetamine, induces an increment in locomotor behavior in rats and this manic-like behavior was prevented by lithium.

However, other factors are also involved in the manic-like behavior induced by methylphenidate, such as oxidative stress. Burrows et al. (2000) showed that the administration of psychostimulants that increase dopaminergic neurotransmission can also cause increase in oxidative stress. Shanthakumar et al. (2013) showed that mice treated with methylphenidate showed

hyperlocomotion and increased oxidative stress, which were blocked by treatment with lithium.

The main antioxidant molecule in the brain is GSH and it participates in many cellular reactions. GSH is involved in the regulation of lipid, glucose and aminoacid utilization. GSH is also involved in several other chemical reactions regarding liver functions, immunity, cell physiology and biosignalling pathways. However, the main activity of GSH is its antioxidant effects. GSH effectively scavenges free radicals and other reactive oxygen species, removing hydrogen and lipid peroxides and preventing oxidation of biomolecules (Wu et al., 2004). This indicates that GSH has extremely important functions. Therefore, depletion of GSH levels may induce serious dysfunctions. Gawryluk et al. (2011) showed that post-mortem prefrontal cortex of BD patients presented diminished levels of GSH compared to brain samples of individuals with no psychiatric illnesses. The study of Macêdo et al. (2013) showed that the administration of lisdexamfetamine dimesylate not only induced manic-like behavior in rats, but also induced decreased GSH content in the PFC, hippocampus and striatum of rats, which was reversed by lithium.

Overall, in our study, methylphenidate increased GSH levels in the PFC and hippocampus, but decreased GSH levels in the striatum. Pearson's correlation showed a negative correlation between hyperlocomotion and GSH levels in the striatum, regarding all animals of the chronic treatment protocol. The heterogeneity concerning the effects of methylphenidate on GSH levels is not abnormal or uncommon, as other research groups have already demonstrated quite diverse results regarding the effect of drugs depending on the dose of the drug, the brain site, the oxidative stress parameter in study, among others (Shanthakumar et al., 2013; Jornada et al., 2011; Bhalla and Dhawan, 2009; Frey et al., 2006). Our resuts showed that quercetin did not reverse methylphenidate-induced decrease in GSH levels, although, in some cases, quercetin increased GSH levels when compared to the control (veh + veh) group.

Emerging data indicates that oxidative stress is present and may play an active role in psychiatric illnesses, including BD. Low levels of free radicals or reactive oxygen/nitrogen species are considered to be normal, but high levels can damage and oxidize nucleic acids, carbohydrates and lipids (Joshi and Praticò, 2014). Given the fact that lipids are the major components of cell membranes, including neuronal membranes, their peroxidation and alterations, which generate hydroperoxides, can greatly affect brain function (Joshi and Praticò, 2014). Studies show that LPO levels are increased during manic episodes and that one of the effects of lithium, the election treatment for mania in BD, include inhibition of LPO and protein oxidation, suggesting that lithium has neuroprotective effects against oxidative stress and that this effect may be related to its antimanic effect (Cui et al., 2007). LPO occuring as a result of uncompensated oxidative stress is an important finding in BD regardless of the stage of the illness (Andreazza et al., 2007; Machado-Vieira et al., 2007). Macêdo et al (2013) also showed increased LPO levels in the PFC, hippocampus and striatum of rats submitted to the animal model of psychostimulant-induced hyperlocomotion. Treatment with lithium was capable of blocking this increase in oxidative stress.

In our study, the effects of methylphenidate administration on oxidative stress were heterogeneous. Pearson's correlation showed a positive correlation between hyperlocomotion and LPO levels in the PFC and hippocampus. Overall, methylphenidate administration increased LPO levels in the PFC, hippocampus and striatum. Acute administration of lithium blocked methylphenidate-induced increase in LPO only in the hippocampus. Acute administration of diazepam was able to block methylphenidate-induced increase in LPO in the hippocampus. Chronic treatment with 10 mg/kg quercetin blocked methylphenidate-induced increase in LPO in the PFC. Chronic treatment with lithium, diazepam and all doses of quercetin were able to block methylphenidate-induced increase in LPO in the striatum. These effects show that quercetin exerted antioxidant effects in the present study.

Arunagiri et al. (2014) showed that methylphenidate treatment decreased GSH levels and increased the levels of LPO in whole brain homogenates, and treatment with lithium restored GSH levels and reduced LPO levels. However, various other studies showed heterogenous results concerning the effects of drugs on brain oxidative stress (Shanthakumar et al., 2013; Jornada et al., 2011; Bhalla and Dhawan, 2009; Frey et al., 2006). Results vary depending on the treatment protocol, on the drug and its doses, on the brain site, among other factors. In the study of Martins et al. (2006), methylphenidate administration reduced LPO levels in the prefrontal cortex and striatum of rats. In lower doses, methylphenidate decreased LPO levels in the hippocampus, but in higher doses, methylphenidate increased LPO levels in this brain site. Therefore, although our study presents heterogeneous results, it reinforces the putative role of oxidative stress in mania.

Also, studies show that the increase in dopaminergic transmission and consequent hyperlocomotion envolve activation of signaling pathways related to enzymes such as PKC (Abrial et al., 2015). Both lithium and quercetin exert antioxidant effects and both are known to possess inhibitory effects on the activity of PKC (Manji and Lenox, 2000). Our research group showed that myricitrin, a flavonoid that possesses antioxidant and PKC-inhibitory effects, was capable of blocking oxidative stress and manic-like behavior (Pereira et al., 2014; Andreatini et al., 2013). Considering that the pathophysiology of mania in BD involves increased PKC activity and oxidative stress (Andreazza et al., 2007; Machado-Vieira et al., 2007; Friedman et al., 1993), we hypothesized that quercetin might possess antimanic properties. Indeed, chronic treatment with 10 mg/kg or 40 mg/kg quercetin blocked methylphenidate-induced hyperlocomotion and in some cases, quercetin was able to restore GSH or decrease LPO levels after methylphenidate administration, like lithium. This indicates that the antimanic-like effect of quercetin may be linked to the decrease in oxidative stress.

5. Conclusions

Chronic treatment with quercetin blocked methylphenidate-induced hyperlocomotion in mice, which denotes an antimanic-like effect of quercetin. Moreover, chronic treatment with quercetin also blocked methylphenidate-induced increase in oxidative stress indexes. These results reveal that quercetin may have an antimanic-like associated with its antioxidant effect, suggesting that quercetin can be an interesting agent in the research of new antimanic drugs.

Contributors

Roberto Andreatini and Inara M.R. Barcaro proposed the study; Luiz K.S. Kanazawa, Débora Dalla Vecchia, Etiéli Mara Wendler, Palloma A.S. Hocayen, Paulo Sérgio Beirão Júnior e Manuela Lima de Mélo conducted the behavioral tests; Luiz K.S. Kanazawa, Francislaine A.R. Lívero, Maria Carolina Stipp and Alexandra Acco were responsible for the evaluation of oxidative stress; all authors contributed for the data analysis and interpretation, wrote the manuscript and approved the final article.

Conflicts of interest

All of the authors declare that they have no conflicts of interest.

Acknowledgements

RA and AA are recipients of a research fellowship from CNPq; LKSK, DDV, EMW, PASH, FARL, MCS and IMRB are recipients of a graduate fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Abrial, E.; Bétourné, A.; Etiévant, A.; Lucas, G.; Scarna, H.; Lambás-Señas, L.; Haddjeri, N. Protein kinase C inhibition rescues manic-like behaviors and hippocampal cell proliferation deficits in the sleep deprivation model of mania. **International Journal of Neuropsychopharmacology**. 18(2); pii: pyu031, 2015.

Andreatini, R.; Pereira, M.; Siba, I. P.; Pizzolatti, M. G.; Santos, A. R. S.; Ruani, A. P. P.2.C.005 Myricitrin, a PKC inhibitor, exerts antimanic-like effects in animal models. **European Neuropsychopharmacology**. 23(2); S357 – S358, 2013.

Andreazza, A. C.; Kauer-Sant'anna, M.; Frey, B. N.; Bond, D. J.; Kapczkinski, F.; Young, L. T.; Yatham, L. N. Oxidative stress markers in bipolar disorder: a meta-analysis. **Journal of Affective Disorders**. 111(2-3); 135 – 44, 2008.

Andreazza, A. C.; Cassini, C.; Rosa, A. R.; Leite, M. C.; de Almeida, L. M.; Nardin, P.; Cunha, A. B.; Ceresér, K. M.; Santin, A.; Gottfried, C.; Salvador, M.; Kapczinski, F.; Gonçalves, C. A. Serum 100B and antioxidant enzymes in bipolar patients. **Journal of Pyschiatric Research**. 41(6); 523 – 9, 2007.

Arunagiri, P.; Rajeshwaran, K.; Shanthakumar, J.; Tamilselvan, T.; Balamurugan, E. Combination of omega-3 fatty acids, lithium, and aripiprazole reduces oxidative stress in brain of mice with mania. **Biological Trace Element Research**. 160(3); 409 – 417, 2014.

Berk, M.; Kapczinski, F.; Andreazza, A. C.; Dean, O. M.; Giorlando, F.; Maes, M.; Yücel, M.; Gama, C. S.; Dodd, S.; Dean, B.; Magalhães, P. V. S.; Amminger, P.; McGorry, P.; Malhi, G. S. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. **Neuroscience and Biobehavioral Reviews**. 35(3); 804 – 817, 2011.

Bhalla, P.; Dhawan, D. K. Protective role of lithium in ameliorating the aluminium-induced oxidative stress and histological changes in rat brain. **Cellular and Molecular Neurobiology**. 29(4); 513 – 521, 2009.

Bhutada, P.; Mundhada, Y.; Bansod, K.; Ubgade, A.; Quazi, M.; Umathe, S.; Mundhada, D. Reversal by quercetin of corticotrophin releasing factor induced anxiety- and depression-like effect in mice. **Progress in Neuro-psychopharmacology & Biological Psychiatry**. 34(6); 955 – 960, 2010.

Burrows, K. B.; Gudelsky, G.; Yamamoto, B. K. Rapid and transient inhibition of mitochondrial function following methamphetamine or 3,4-

methylenedioxymethamphetamine administration. **European Journal of Pharmacology**. 398(1); 11 – 18, 2000.

Cui, J.; Shao, L.; Young, L. T.; Wang, J. F. Role of glutathione in neuroprotective effects of mood stabilizing drugs lithium and valproate. **Neuroscience**. 144(4); 1447 – 1453, 2007.

Dajas, F. Abin-Carriquiry, J. A.; Arredondo, F.; Blasina, F.; Echeverry, C.; Martínez, M.; Rivera, F.; Vaamonde, L. Quercetin in brain diseases: potential and limits. **Neurochemistry International**. 89; 140 – 148, 2015.

Dziedzicka-Wasylewska, M.; Mackowiak, M.; Fijat, K.; Wedzony, K. Adaptive changes in the rat dopaminergic transmission following repeated lithium administration. **Journal of Neural Transmission**. 103(7); 765 – 776, 1996.

Einat, H. Modelling facets of mania – new directions related to the notion of endophenotypes. **Journal of Psychopharmacology**. 20(5); 714 – 22, 2006.

Friedman, E.; Wang, H. Y.; Levinson, D.; Connell, T. A.; Singh, H. Altered platelet protein kinase C activity in bipolar affective disorder, manic episode. **Biological Psychiatry**. 33(7); 520 – 525, 1993.

Frey, B. N.; Valvassori, S. S.; Réus, G. Z.; Martins, M. R.; Petronilho, F. C.; Bardini, K.; Dal-Pizzol, F.; Kapczinski, F.; Quevedo, J. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. **Reviews on Psychiatric Neuroscience**. 31(5); 326 – 332, 2006.

Gamet-Payrastre, L.; Manenti, S.; Gratacap, M. P.; Tulliez, J.; Chap, H.; Payrastre, B. Flavonoids and the inhibition of PKC and PI 3-kinase. **General Pharmacology**. 32(3); 279 – 286, 1999.

Gawryluk, J. W.; Wang, J. F.; Andreazza, A. C.; Shao, L.; Young, L. T. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. **International Journal of Neuropsychopharmacology**. 14(1); 123 – 130, 2011.

Gould, T. J.; Keith, R. A.; Bhat, R. V. Differential sensitivity to lithium's reversal of amphetamine-induced open-field activity in two inbred strains of mice. **Behavioural Brain Research**. 118(1); 95 – 105, 2001.

Hoertel, N.; de Maricourt, P.; Gorwood, P. Novel routes to bipolar disorder drug discovery. **Expert Opinion On Drug Discovery**. 8(8); 907 – 18, 2013.

Jacobson, D.; Silverstone, T. Dextroamphetamine-induced arousal in human subjects as a model of mania. **Psychological Medicine**. 16(2); 323 – 329, 1986.

Jiang, Z. Y.; Hunt, J. V.; Wolff, S. P. Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. **Analytical Biochemistry**. 202(2); 384–389, 1992.

Jornada, L. K.; Valvassori, S. S.; Steckert, A. V.; Moretti, M.; Mina, F.; Ferreira, C. L.; Arent, C. O.; Dal-Pizzol, F.; Quevedo, J. Lithium and valproate modulate antioxidant enzymes and prevent ouabain-induced oxidative damage in an animal model of mania. **Journal of Psychiatric Research**. 45(2); 162 – 168, 2011.

Joshi, Y. B.; Praticò, D. Lipid peroxidation in psychiatric illness: overview of clinical evidence. **Oxidative Medicine and Cellular Longevity**. 2014.

Machado-Vieira, R.; Andreazza, A. C.; Viale, C. I.; Zanatto, V.; Ceresér, V. Jr.; da Silva Vargas, R.; Kapczinski, F.; Portela, L. V.; Souza, D. O.; Salvador, M.; Gentil, V. Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects. **Neuroscience Letters**. 421(1); 33 – 6, 2007.

Machado-Vieira, R.; Kapczinski, F.; Soares, J. C. Perspectives for the development of animal models of bipolar disorder. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**. 28(2); 209 – 224, 2004.

Macêdo, D. S.; de Lucena, D. F.; Queiroz, A. I. G.; Cordeiro, R. C.; Araújo, M. M.; Sousa, F. C.; Vasconcelos, S. M.; Hyphantis, T. N.; Quevedo, J.; McIntyre, R. S.; Carvalho, A. F. Effects of lithium on oxidative stress and behavioral alterations induced by lisdexamfetamine dimesylate: Relevance as an animal model of mania. **Progress In Neuro-Psychopharmacology & Biological Psychiatry**. 43; 230 – 237, 2013.

Manji, H. K.; Lenox, R. H. Signaling: cellular insights into the pathophysiology of bipolar disorder. **Biological Psychiatry**. 48; 518 – 530, 2000.

Martins, M. R.; Reinke, A.; Petronilho, F. C.; Gomes, K. M.; Dal-Pizzol, F.; Quevedo, J. Methylphenidate treatment induces oxidative stress in young rat brain. **Brain Research**. 1078(1); 189 – 197, 2006.

Mines, M. A.; Beurel, E.; Jope, R. S. Examination of methylphenidate-mediated behavior regulation by glycogen synthase kinase-3 in mice. **European Journal of Pharmacology**. 698(1-3); 252 – 258, 2013.

Nivoli, A. M. A.; Murru, A.; Goikolea, J. M.; Crespo, J. M.; Montes, J. M.; González-Pinto, A.; García-Portilla, P.; Bobes, J.; Sáiz-Ruiz, J.; Vieta, E. New treatment guidelines for acute bipolar mania: a critical review. **Journal of Affective Disorders**. 140(2); 125 – 141, 2012.

Pereira, M.; Andreatini, R.; Schwarting, R. K.; Brenes, J. C. Amphetamine-induced appetitive 50-kHz calls in rats: a marker of affect in mania? **Psychopharmacology**. 231(13); 2567 - 77, 2014.

Pereira, M.; Martynhak, B. J.; Baretta, I. P.; Correia, D.; Siba, I. P.; Andreatini, R. Antimanic-like effect of tamoxifen is not reproduced by acute or chronic administration of medroxyprogesterone or clomiphene**. Neuroscience Letters**. 500(2); 95 – 98, 2011.

Post, R. M.; Jimerson, D. C.; Bunney, W. E.; Goodwin, F. K. Dopamine and mania: behavioural and biochemical effects of the dopamine receptor blocker pimozide. **Psychopharmacology**. 67(3); 297 – 305, 1980.

Sabioni, P.; Baretta, I. P.; Ninomiya, E. M.; Gustafson, L.; Rodrigues, A. L.; Andreatini, R. The antimanic-like effect of tamoxifen: Behavioral comparison with other PKC-inhibiting and antiestrogenic drugs. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**. 32(8); 1927-1931, 2008.

Schuepbach, D. Pharmacotherapy of bipolar disorder and acute mania: the clinical context of selected guidelines. **European Psychiatry**. 30(Suppl. 1); 1135, 2015.

Sedlak, J.; Lindsay, R. H. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. **Analytical Biochemistry**. 25(1); 192 – 205, 1968.

Shanthakumar, J.; Tamilselvan, T.; Arunagiri, P.; Rajeshwaran, K.; Balamurugan, E. Role of caffeine in lithium treated methylphenidate induced oxidative stress in an animal model of mania. **Asian Journal of Pharmaceutical Sciences**. 3(4); 166 - 171, 2013.

Wang, H. Y.; Friedman, E. Lithium inhibition of protein kinase C activation-induced serotonin release. **Psychopharmacology**. 99(2); 213 – 218, 1989.

Wu, G.; Fang, Y. Z.; Yang, S.; Lupton, J. R.; Turner, N. D. Glutathione metabolism and its implications for health. **Journal of Nutrition**. 134(3); 489 – 492, 2004.

Young, J. W.; Henry, B. L.; Geyer, M. A. Predictive animal models of mania: hits, misses and future directions. **British Journal of Pharmacology**. 164(4); 1263 – 1284, 2011.

Young, J. W.; Minassian, A.; Paulus, M. P.; Geyer, M. A.; Perry, W. A reverse-translational approach to bipolar disorder: rodent and human studies in the behavioral pattern monitor. **Neuroscience & Biobehavior Reviews**. 31(6); 882 – 896, 2007.

5 CONSIDERAÇÕES FINAIS

Levando em consideração os problemas relacionados ao tratamento farmacológico do TB, faz-se necessário a realização de pesquisas científicas para a descoberta de novos agentes terapêuticos. A fisiopatologia da mania no TB envolve diversos fatores, dentre eles a presença de estresse oxidativo (BERK et al., 2011; ANDREAZZA et al., 2007; MACHADO-VIEIRA et al., 2007) e o aumento da atividade da enzima PKC (EINAT et al., 2007; WANG; FRIEDMAN, 1996). O lítio, tratamento de primeira linha para o TB, apresenta diversas propriedades, como a capacidade de combater o estresse oxidativo (JORNADA et al., 2011) e de inibir a atividade da enzima PKC (MANJI; LENOX, 1999). O flavonoide quercetina, por apresentar ação antioxidante (DAJAS et al., 2015) e também possuir atividade inibitória sobre a PKC (GAMET-PAYRASTRE, 1999), poderia também apresentar um efeito tipo antimaníaco.

Os tratamentos com quercetina e com lítio foram capazes de reverter o comportamento tipo maníaco (hiperlocomoção) em dois modelos animais de mania: hiperlocomoção induzida por PSD e por metilfenidato. O primeiro modelo é um modelo não-farmacológico e o segundo, um modelo farmacológico de indução de comportamento tipo maníaco. Um dos mecanismos pelos quais tais modelos induzem hiperlocomoção pode ser por alterações na neurotransmissão dopaminérgica (GESSA et al., 1995). A privação de sono está relacionada a uma diminuição na neurotransmissão dopaminérgica, que, por sua vez, está relacionada a uma diminuição da expressão de TH em neurônios da via nigroestriatal (LIMA et al., 2012). Isso leva a um aumento da sensibilidade de receptores dopaminérgicos e maior estímulo da atividade locomotora (LIMA et al., 2007). A privação de sono também estimula a atividade da PKC (ABRIAL et al., 2015), que é capaz de fosforilar o DAT, impedindo a receptação de dopamina. O aumento dos níveis de dopamina estimula aumento da atividade locomotora.

O psicoestimulante metilfenidato também altera a neurotransmissão dopaminérgica por se ligar ao DAT, impedindo sua receptação (GATLEY et al., 1996). Com isso, o aumento da atividade dopaminérgica estimula o aumento da atividade locomotora. O metilfenidato também estimula a atividade da PKC (BARBOSA et al., 2011), que está envolvida na fosforilação do DAT, o que também impede a receptação de dopamina.

Um dos mecanismos pelos quais a quercetina e o lítio podem atuar diminuindo a hiperlocomoção induzida por ambos os modelos animais de mania pode ser a inibição da atividade da PKC, o que impede a fosforilação do DAT e, com isso, ocorre uma diminuição na neurotransmissão dopaminérgica e diminuição da hiperlocomoção. No entanto, outros mecanismos, além do aumento da atividade da PKC, estão envolvidos no contexto da neurotransmissão dopaminérgica e aumento da atividade locomotora. Portanto, a quercetina e o lítio provavelmente atuam também por outros mecanismos.

Além disso, no geral, em ambos os modelos, houve o estabelecimento de estresse oxidativo, decorrentes de alterações nos níveis de GSH ou aumento de níveis de LPO no PFC, hipocampo e estriado dos animais. Em alguns casos, os níveis de GSH foram reduzidos, o que denota a depleção de uma molécula antioxidante, podendo caracterizar estresse oxidativo (GAWRYLUK et al., 2011). Em outros casos, os níveis de GSH encontravam-se aumentados, o que pode ser decorrente de um mecanismo compensatório do organismo no intuito de combater o excesso de radicais livres, EROs ou ERNs (CHINTA et al., 2006). Em ambos os casos, pode-se deduzir que houve estresse oxidativo.

Os tratamentos com quercetina e com lítio, no geral, foram capazes de reverter o estresse oxidativo gerado pela privação de sono e pela administração de metilfenidato.

No geral, a quercetina foi capaz de reverter o estresse oxidativo e a hiperlocomoção nos dois modelos de mania, apresentando efeito antioxidante e também efeito tipo antimaníaco.

6 CONCLUSÃO

O presente estudo mostrou que a administração de quercetina foi capaz de reduzir a hiperlocomoção induzida por PSP (24 h) e metilfenidato em camundongos. Este efeito tipo antimaníaco da quercetina parece estar relacionado ao seu efeito antioxidante. No geral, ambos os efeitos da quercetina foram similares aos do lítio, droga antimaníaca utilizada na clínica. O diazepam não apresentou efeito antioxidante, nem antimaníaco, mas sim sedativo.

Portanto, este estudo mostra que a quercetina, além de efeito antioxidante, possui um potencial efeito antimaníaco. Contudo, são necessários estudos adicionais para a elucidação dos mecanismos de ação da quercetina.

REFERÊNCIAS

ABRIAL, E.; BÉTOURNÉ, A.; ETIÉVANT, A.; LUCAS, G.; SCARNA, H.; LAMBÁS-SEÑAS, L.; HADDJERI, N. Protein kinase C inhibition rescues manic-like behaviors and hippocampal cell proliferation deficits in the sleep deprivation model of mania. **International Journal of Neuropsychopharmacology**. 18(2); pii: pyu031, 2015.

ABRIAL, E.; LUCAS, G.; SCARNA, H.; HADDJERI, N.; LAMBÁS-SEÑAS, L. A role for the PKC signaling system in the pathophysiology and treatment of mood disorders: involvement of a functional imbalance? **Molecular Neurobiology**. 44(3); 407 – 419, 2011.

AGULLO, G.; GAMET-PAYRASTRE, L.; MANENTI, S.; VIALA, C.; RÉMÉSY, C.; CHAP, H.; PAYRASTRE, B. Relationship between flavonoid structure and inhibition of phosphatidylinositol 3-kinase: a comparison with tyrosine kinase and protein kinase C inhibition. **Biochemical Pharmacology**. 53(11); 1649 – 57, 1997.

AMERICAN PSYCHIATRY ASSOCIATION. Diagnostic and Statistical Manual of Mental Disorders. **5ª ed**. Washington, DC: American Psychiatry Association, 2013.

ANAND, A.; VERHOEFF, P.; SENECA, N.; ZOGHBI, S. S.; SEIBYL, J. P.; CHARNEY, D. S.; INNIS, R. B. Brain SPECT imaging of amphetamine-induced dopamine release in euthymic bipolar disorder patients. **American Journal of Psychiatry**. 157(7); 1108 – 1114, 2000.

ANDREAZZA, A. C.; KAPCZINSKI, F.; KAUER-SANT'ANNA, M.; WALZ, J. C.; BOND, D. J.; GONÇALVES, C. A.; YOUNG, L. T.; YATHAM, L. N. 3-Nitrotyrosine and glutathione antioxidant system in patients in the early and late stages of bipolar disorder. **Journal of Psychiatry & Neuroscience**. 34 (4); 263 – 271, 2009.

ANDREAZZA, A. C.; KAUER-SANT'ANNA, M.; FREY, B. N.; BOND, D. J.; KAPCZINSKI, F.; YOUNG, T.; YATHAM, L. N. Oxidative stress markers in bipolar disorder: a meta-analysis. **Journal of Affective Disorders**. 111(2-3); 135-144, 2008.

ANDREAZZA, A. C.; CASSINI, C.; ROSA, A. R.; LEITE, M. C.; de ALMEIDA, L. M.; NARDIN, P.; CUNHA, A. B.; CERESÉR, K. M.; SANTIN, A.; GOTTFRIED, C.; SALVADOR, M.; KAPCZINSKI, F.; GONÇALVES, C. A. Serum 100B and antioxidant enzymes in bipolar patients. **Journal of Pyschiatric Research**. 41(6); 523 – 9, 2007.

BARBOSA, F. J.; HESSE, B.; de ALMEIDA, R. B.; BARETTA, I. P.; BOERNGEN-LACERDA, R.; ANDREATINI, R. Magnesium sulfate and sodium valproate block methylphenidate-induced hyperlocomotion, an animal model of mania. **Pharmacological Reports**. 63(1); 64-70, 2011.

BERK, M.; KAPCZINSKI, F.; ANDREAZZA, A. C.; DEAN, O. M.; GIORLANDO, F.; MAES, M.; YÜCEL, M.; GAMA, C. S.; DODD, S.; DEAN, B.; MAGALHÃES, P. V. S.; AMMINGER, P.; MCGORRY, P.; MALHI, G. S. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. **Neuroscience and Biobehavioral Reviews**. 35(3); 804-817, 2011.

BERK, M.; DODD, S.; KAUER-SANT'ANNA, M.; MALHI, G. S.; BOURIN, M.; KAPCZINSKI, F.; NORMAN, T. Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. **Acta Psychiatrica Scandinavica Supplementum**. 434; 41-49, 2007.

BENES, F. M.; BERETTA, S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. **Neuropsychopharmacology**. 25(1); 1 – 27, 2001.

BHALLA, P.; DHAWAN, D. K. Protective role of lithium in ameliorating the aluminium-induced oxidative stress and histological changes in rat brain. **Cellular and Molecular Neurobiology**. 29(4); 513 – 521, 2009.

BHUTADA, P.; MUNDHADA, Y.; BANSOD, K.; UBGADE, A.; QUAZI, M.; UMATHE, S.; MUNDHADA, D. Reversal by quercetin of corticotrophin releasing factor induced anxiety- and depression-like effect in mice. **Progress in Neuro-psychopharmacology & Biological Psychiatry**. 34(6); 955 – 960, 2010.

BOOTS, A. W.; HAENEN, G. R. M. M.; BAST, A. Health effects of quercetin: from antioxidant to nutraceutical. **European Journal of Pharmacology**. 585(2-3); 325 – 37, 2008.

BRÜNING, C. A.; PRIGOL, M.; LUCHESE, C.; PINTON, S.; NOGUEIRA, C. W. Diphenyl diselenide ameliorates behavioral and oxidative parameters in an animal model of mania induced by ouabain. **Progress in Neuropsychopharmacology and Biological Psychiatry**. 38(2); 168-174, 2012.

CASTRO-COSTA, E.; SILVA, A. G. A adesão terapêutica no transtorno bipolar. **Revista Debates em Psiquiatria**. 1(5); 5, 2011.

CHINTA, S. J.; KUMAR, J. M.; ZHANG, H.; FORMAN, H. J.; ANDERSEN, J. K. Up-regulation of gamma-glutamyl transpeptidase activity following glutathione depletion has a compensatory rather than an inhibitory

effect on mitochondrial complex I activity: implications for Parkinson's disease. **Free Radical Biology & Medicine**. 40(9); 1557 – 1563, 2006.

CHIU, C. T.; WANG, Z.; HUNSBERGER, J. G.; CHUANG, D. M. Therapeutic potential of mood stabilizers lithium and valproic acid: beyond bipolar disorder. **Pharmacological Reviews**. 65(1); 105 – 142, 2013.

COLOMBO, C.; BENEDETTI, F.; BARBINI, B.; CAMPORI, E.; SMERALDI, E. Rate of switch from depression into mania after therapeutic sleep deprivation in bipolar depression. **Psychiatry Research**. 86(3); 267 – 270, 1999.

COMALADA, M.; CAMUESCO, D.; SIERRA, S.; BALLESTER, I.; XAUS, J.; GÁLVEZ, J.; ZARZUELO, A. *In vivo* quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-κB pathway. **European Journal of Immunology**. 35(2); 584 – 592, 2005.

DAJAS, F.; ABIN-CARRIQUIRY, J. A.; ARREDONDO, F.; BLASINA, F.; ECHEVERRY, C.; MARTÍNEZ, M.; RIVERA, F.; VAAMONDE, L. Quercetin in brain diseases: potential and limits. **Neurochemistry International**. 89; 140-148, 2015.

DAJAS, F. Life or death: neuroprotective and anticancer effects of quercetin. **Journal of Ethnopharmacology**. 143(2); 383 – 96, 2012.

D'AQUILA, P. S.; COLLU, M.; GESSA, G. L.; SERRA, G. The role of dopamine in the mechanism of action of antidepressant drugs. **European Journal of Pharmacology**. 405(1-3); 365 – 373, 2000.

DA SILVA, A. G.; BUENO, J. R.; MORENO, R. A. Transtorno Bipolar. **Revista Debates em Psiquiatria**. 1(5); 5, 2011.

DE GROOT, H. Reactive oxygen species in tissue injury. **Hepatogastroenterology**. 41(4); 328-332, 1994.

DE LA LASTRA, C. A.; CALERO, M. J. M.; MOTILVA, V. Antiulcer and gastroprotective effects of quercetin: a gross and histologic study. **Pharmacology**. 48(1); 56 – 62, 1994.

DI CHIARA, G. Psychobiology of the role of dopamine in drug-abuse and addiction. **Neuroscience Research Community**. 17; 133 – 143, 1995.

DIPLOCK, A. T.; CHARLEUX, J. L.; CROZIER-WILLI, G.; KOK, F. J.; RICE-EVANS, C.; ROBERFROID, M.; STAHL, W.; VIÑA-RIBES, J. Functional

food science and defence against reactive oxidative stress. **British Journal of Nutrition**. 80(1); S77 – 112, 1998.

ECHEVERRY, C.; ARREDONDO, F.; ABIN-CARRIQUIRY, J. A.; MIDIWO, J. O.; OCHIENG, C.; KERUBO, L.; DAJAS, F. Pretreatment with natural flavones and neuronal cell survival after oxidative stress: a structure-activity relationship study. **Journal of Agricultural and Food Chemistry**. 58(4); 2111 – 2115, 2010.

EINAT, H.; YUAN, P.; SZABO, S. T.; DOGRA, S.; MANJI, H. K. Protein kinase C inhibition by tamoxifen antagonizes manic-like behavior in rats: implications for the development of novel therapeutics for bipolar disorder. **Neuropsychobiology**. 55(3-4); 123-31, 2007.

EINAT, H. Modelling facets of mania: new directions related to the notion of endophenotypes. **Journal of Psychopharmacology**. 20(5); 714-722, 2006.

FELDMAN-NAIM, S.; TURNER, E. H.; LEIBENLUFT, E. Diurnal variation in the direction of mood switches in patients with rapid-cycling bipolar disorder. **Journal of Clinical Psychiatry**. 58(2); 79 – 84, 1997.

FILHO, A. W.; FILHO, V. C.; OLINGER, L.; DE SOUZA, M. M. Quercetin: further investigation of its antinociceptive properties and mechanisms of action. **Archives of Pharmacal Research**. 31(6); 713 – 721, 2008.

FLAISHER-GRINBERG, S.; KRONFELD-SCHOR, N.; EINAT, H. Models of mania: from facets to domains and from animal models to model animals. **Journal of Psychopharmacology**. 24(3); 437 – 438, 2010.

FRIEDMAN, E.; WANG, H. Y.; LEVINSON, D.; CONNELL, T. A.; SINGH, H. Altered platelet protein kinase C activity in bipolar affective disorder, manic episode. **Biological Psychiatry**. 33(7); 520 – 525, 1993.

GAMET-PAYRASTRE, L.; MANENTI, S.; GRATACAP, M. P.; TULLIEZ, J.; CHAP, H.; PAYRASTRE, B. [Flavonoids and the inhibition of PKC and PI 3-kinase. **General Pharmacology**. 32(3); 279-286, 1999.

GATLEY, S. J.; PAN, D.; CHEN, R.; CHATURVEDI, G.; DING, Y. S. Affinities of methylphenidate derivatives for dopamine, norepinephrine and serotonin transporters. **Life Sciences**. 58(12); 231 – 39, 1996.

GEDDES, J. R.; MIKLOWITZ, D. J. Treatment of bipolar disorder. **Lancet**. 381(9878); 1672 – 82, 2013.

GESSA, G. L.; PANI, L.; FADDA, P.; FRATTA, W. Sleep deprivation in the rat: an animal model of mania. **European Neuropsychopharmacology**. 5(Suppl.); 89-93, 1995.

GOULD, T. D.; O'DONNELL, K. C.; PICCHINI, A. M.; MANJI, H. K. Strain differences in lithium attenuation of D-amphetamine –induced hyperlocomotion: a mouse model for the genetics of clinical response to lithium. **Neuropsychopharmacology**. 32(6); 1321 – 1333, 2007.

GOULD, T. D.; PICCHINI, A. M.; EINAT, H.; MANJI, H. K. Targeting glycogen synthase kinase-3 in the CNS: implications for the development of new treatments for mood disorders. **Current Drug Targets**. 7(11); 1399 – 1409, 2006.

HALLIWELL, B. Antioxidant characterization: Methodology and mechanism. **Biochemical Pharmacology**. 49(10); 1341 – 1348, 1995.

HARVEY, A. G. Sleep and circadian rhythms in bipolar disorder: seeking synchrony, harmony, and regulation. **American Journal of Psychiatry**. 165(7); 820 – 829, 2008.

HUDSON, J. I.; LIPINSKI, J. F.; FRANKENBURG, F. R.; TOHEN, M.; KUPFER, D. J. Effects of lithium on sleep in mania. **Biological Psychiatry**. 25(5); 665 – 668, 1989.

HUDSON, J. I.; LIPINSKI, J. F.; FRANKENBURG, F. R.; GROCHOCINSKI, V. J.; KUPFER, D. J. Electroencephalographic sleep in mania. **Archives of General Psychiatry**. 45(3); 267 – 273, 1988.

JORNADA, L. K.; VALVASSORI, S. S.; STECKERT, A. V.; MORETTI, M.; MINA, F.; FERREIRA, C. L.; ARENT, C. O. DAL-PIZZOL, F.; QUEVEDO, J. Lithium and valproate modulate antioxidant enzymes and prevent ouabain-induced oxidative damage in an animal model of mania. **Journal of Psychiatric Research**. 45(2); 162 – 168, 2011.

KEMP, D. E.; SYLVIA, L. G.; CALABRESE, J. R.; NIERENBERG, A. A.; THASE, M. E.; REILLY-HARRINGTON, N. A.; OSTACHER, M. J.; LEON, A. C.; KETTER, T. A.; FRIEDMAN, E. S.; BOWDEN, C. K.; PENCINA, M.; IOSIFESCU, D. V. General medical burden in bipolar disorder: findings from the LiTMUS comparative effectiveness trial. **Acta Psychiatrica Scandinavica**. 129(1); 24-34, 2014.

KOHEN, R.; NYSKA, A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox actions, and methods for their quantification. **Toxicology Pathology**. 30(6); 620 – 650, 2002.

KNAPP, P.; ISOLAN, L. Abordagens psicoterápicas no transtorno bipolar. **Revista de Psiquiatria Clínica**. 32(1); 98 – 104, 2005.

LAFER, B.; NERY, F. G. Tratamento da depressão bipolar. **Revista Debates em Psiquiatria**. 1(5); 5, 2011.

LAHERA, G.; FREUND, N.; SÁIZ-RUIZ, J. Salience and dysregulation of the dopaminergic system. **Revista de Psiquiatria y Salud Mental**. 6(1); 45 – 51, 2013.

LAKHANPAL, P.; RAI, D. K. Quercetin: a versatile flavonoid. **Internet Journal of Medical Update**. 2(2); 22 – 37, 2007.

LIMA, M. M. S.; ANDERSEN, M. L.; REKSIDLER, A. B.; FERRAZ, A. C.; VITAL, M. A. B. F.; TUFIK, S. Paradoxical sleep deprivation modulates tyrosine hydroxylase expression in the nigrostriatal pathway and attenuates motor deficits induced by dopaminergic depletion. **CNS & Neurological Disorders – Drug Targets**. 11(4); 359 – 368, 2012.

LIMA, M. M. S.; ANDERSEN, M. L.; REKSIDLER, A. B.; VITAL, M. A. B. F.; TUFIK, S. The role of the substantia nigra pars compacta in regulating sleep patterns in rats. **PLoS ONE**. 2(6): e513, 2007.

LÓPEZ-FIGUEROA, A. L.; NORTON, C. S.; LÓPEZ-FIGUEROA, M. O.; ARMELLINI-DODEL, D.; BURKE, S.; AKIL, H.; LÓPEZ, J. F.; WATSON, S. J. Serotonin 5-HT1A, 5-HT1B, and 5-HT2A receptor mRNA expression in subjects with major depression, bipolar disorder, and schizophrenia. **Biological Psychiatry**. 55(3); 225 – 233, 2004.

MACHADO-VIEIRA, R.; ANDREAZZA, A. C.; VIALE, C. I.; ZANATTO, V.; CERESÉR, V. JR.; DA SILVA VARGAS, R.; KAPCZINSKI, F.; PORTELA, L. V.; SOUZA, D. O.; SALVADOR, M.; GENTIL, V. = Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects. **Neuroscience Letters**. 421(1); 33 – 6, 2007.

MANJI, H. K.; MOORE, G. J.; CHEN, G. Bipolar disorder: leads from the molecular and cellular mechanisms of action of mood stabilisers. **British Journal of Psychiatry**. 178(41); 107 – 119, 2001.

MANJI, H. K.; LENOX, R. H. Signaling: cellular insights into the pathophysiology of bipolar disorder. **Biological Psychiatry**. 48(6); 518-530, 2000.

MANJI, H. K.; LENOX, R. H. Protein kinase C signaling in the brain: molecular transduction of mood stabilization in the treatment of bipolar disorder. **Biological Psychiatry**. 46(10); 1328 – 1351, 1999.

MANSELL, W.; PEDLEY, R. The ascent into mania: a review of psychological processes associated with the development of manic symptoms. **Clinical Psychology Review**. 28(3); 494 – 520, 2008.

MICHAEL, N.; ERFURTH, A.; OHRMANN, P.; GÖSSLING, M.; AROLT, V.; HEINDEL, W.; PRLEIDERER, B. Acute mania is accompanied by elevated glutamate / glutamine levels within the left dorsolateral prefrontal cortex. **Psychopharmacology**. 168(3); 344 – 346, 2003.

MIKLOWITZ, D. J.; JOHNSON, S. L. The psychopathology and treatment of bipolar disorder. **Annual Reviews in Clinical Psychology**. 2(1); 199 – 235, 2006.

MÜLLER-OERLINGHAUSEN, B.; BERGHÖFER, A.; BAUER, M. Bipolar disorder. **Lancet**. 359(9302); 241-247, 2002.

O'NEILL, M. F.; SHAW, G. Comparison of dopamine receptor antagonists on hyperlocomotion induced by cocaine, amphetamine, MK-801 and the dopamine D1 agonist C-APB in mice. **Psychopharmacology**. 145(3); 237 – 250, 1999.

OZCAN, M. E.; GULEC, M.; OZEROL, E.; POLAT, R.; AKYOL, O. Antioxidant enzyme activities and oxidative stress in affective disorders. **International Clinical Psychopharmacology**. 19(2); 89 – 95, 2004.

PARKER, J. P.; MURRAY-RUST, J. PKC at a glance. **Journal of Cell Science**. 117; 131 – 132, 2004.

PEREIRA, M. **Avaliação do efeito tipo antidepressivo e antimaníaco da miricitrina em testes animais**. 76f. Tese (Doutorado em Farmacologia) – Universidade Federal do Paraná (UFPR). Curitiba, 2013.

PILETZ, J. E.; DEMET, E.. GWIRTSMAN, H. E.; HALARIS, A. Disruption of circadian MHPG rhythmicity in major depression. **Biological Psychiatry**. 35 (11); 830 – 842, 1994.

REINARES, M.; ROSA, A. R.; FRANCO, C.; GOIKOLEA, J. M.; FOUNTOULAKIS, K.; SIAMOULI, M.; GONDA, X.; FRANGOU, S.; VIETA, E. A systematic review on the role of anticonvulsants in the treatment of acute bipolar depression. **International Journal of Neuropsychopharmacology**. 16(2); 485 – 96, 2013.

RIGANO, D.; FORMISANO, C.; BASILE, A.; LAVITOLA, A.; SENATORE, F.; BRUNO, M. Antibacterial activity of flavonoids and phenylpropanoids from Marrubium globosum ssp. Libanoticum. **Phytotherapy Research**. 21(4); 395 – 7, 2007.

RINWA, P.; KUMAR, A. Quercetin suppress microglial neuroinflammatory response and induce antidepressent[sic]-like effect in olfactory bulbectomized rats. **Neuroscience**. 255; 86-98, 2013.

ROSA, A. R.; SINGH, N.; WHITAKER, E.; DE BRITO, M.; LEWIS, A. M.; VIETA, E.; CHURCHILL, G. C.; GEDDES, J. R.; GOODWIN, G. M. Altered plasma glutathione levels in bipolar disorder indicates higher oxidative stress; a possible risk factor for illness onset despite normal brain-derived neurotrophic factor (BDNF) levels. **Psychological Medicine**. 44(11); 2409 – 18, 2014.

ROYBAL, K.; THEOBOLD, D.; GRAHAM, A.; DINIERI, J. A.; RUSSO, S. J.; KRISHNAN, V.; CHAKRAVARTY, S.; PEEVEY, J.; OEHRLEIN, N.; BIRNBAUM, S.; VITATERNA, M. H.; ORSULAK, P.; TAKAHASHI, J. S.; NESTLER, E. J.; CARLEZON, W. A.; MCCLUNG, C. A. Mania-like behavior induced by disruption of CLOCK. **PNAS**. 104(15); 6406 – 6411, 2007.

RUSSO, M.; SPAGNUOLO, C.; TEDESCO, I.; BILOTTO, S.; RUSSO, G. L. The flavonoid quercetin in disease prevention and therapy: facts and fancies. **Biochemical Pharmacology**. 83(1); 6 – 15, 2012.

SALVADORE, G.; QUIROZ, J. A.; MACHADO-VIEIRA, R.; HENTER, I. D.; MANJI, H. K.; ZARATE, C. A. The neurobiology of the switch process in bipolar disorder: a review. **Journal of Clinical Psychiatry**. 71(11); 1488 – 1501, 2010.

SERRETTI, A.; OLGIATI, P. Profiles of “manic” symptoms in bipolar I, bipolar II, and major depressive disorders. **Journal of Affective Disorders**. 84(2-3); 159 – 166, 2005.

SHIAH, I. S.; YATHAM, L. N. Serotonin in mania and in the mechanism of action of mood stabilizers: a review of clinical studies. **Bipolar Disorders**. 2(2); 77 – 92, 2000.

SOUZA, F. G. M. Tratamento de manutenção no transtorno bipolar. **Revista Debates em Psiquiatria**. 1(5); 5, 2011.

SULZER, D.; CHEN, T. K.; LAU, Y. Y.; KRISTENSEN, H.; RAYPORT, S.; EWING, A. Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. **Journal of Neuroscience**. 15(5 Pt 2); 4102 – 4108, 1995.

TUFIK, S.; LINDSEY, C. J.; CARLINI, E. A. Does REM sleep deprivation induce a supersensitivity of dopaminergic receptors in the rat brain? **Pharmacology**. 16(2); 98 – 105, 1978.

VAN KAMMEN, D. P.; MURPHY, D. L. Attenuation of the euphoriant and activating effects of D- and L-amphetamine by lithium carbonate treatment. **Psychopharmacologia**. 44(3); 215 – 224, 1975.

WANG, H. Y.; FRIEDMAN, E. Enhanced protein kinase C activity and translocation in bipolar affective disorder brains. **Biological Psychiatry**. 40(7); 568 – 575, 1996.

WANG, H. Y.; FRIEDMAN, E. Lithium inhibition of protein kinase C activation-induced serotonin release. **Psychopharmacology**. 99(2); 213 – 218, 1989.

WAY, K. J.; CHOU, E.; KING, G. L. Identification of PKC-isoform-specific biological actions using pharmacological approaches. **Trends in Pharmacological Sciences**. 21(5); 181 – 187, 2000.

WEHR, T. A.; GOODWIN, F. K.; WIRZ-JUSTICE, A.; BREITMAIER, J.; CRAIG, C. 48-hour sleep-wake cycles in manic-depressive illness: naturalistic observations and sleep deprivation experiments. **Archives of General Psychiatry**. 39(5); 559 – 565, 1982.

WETSEL, W. C.; KHAN, W. A.; MERCHENTHALER, I.; RIVERA, H.; HALPERN, A. E.; PHUNG, H. M.; NEGRO-VILAR, A.; HANNUN, Y. A. Tissue and cellular distribuition of the extended family of protein kinase C isoenzymes. **Journal of Cell Biology**. 117(1); 121 – 133, 1992.

WILLIAMS, R. J.; SPENCER, J. P. E.; RICE-EVANS, C. Flavonoids: antioxidants or signalling molecules? **Free Radicals Biology and Medicine**. 36(7); 838 – 849, 2004.

YOUNG, J. W.; MINASSIAN, A.; PAULUS, M. P.; GEYER, M. A.; PERRY, W. A reverse-translational approach to bipolar disorder: rodent and human studies in the behavioral pattern monitor. **Neuroscience & Biobehavior Reviews**. 31(6); 882 – 896, 2007.

ANEXO 1 – Certificado do Comitê de Ética

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Comissão de Ética no Uso de Animais
(CEUA)

UFPR
UNIVERSIDADE FEDERAL DO PARANÁ

Nº 733

CERTIFICADO

A Comissão de Ética no Uso de Animais (CEUA) do Setor de Ciências Biológicas da Universidade Federal do Paraná, instituído pela PORTARIA Nº 787/03-BL, de 11 de junho de 2003, com base nas normas para a constituição e funcionamento da CEUA, estabelecidas pela RESOLUÇÃO Nº 01/03-BL, de 09 de maio de 2003 e considerando o contido no Regimento Interno da CEUA, **CERTIFICA** que os procedimentos utilizando animais no projeto de pesquisa abaixo especificado, estão de acordo com os princípios éticos estabelecidos pelo Colégio Brasileiro de Experimentação Animal (COBEA) e exigências estabelecidas em "*Guide for the Care and Use of Experimental Animals (Canadian Council on Animal Care)*".

CERTIFICATION

The Ethics Animal Experiment Committee of the Setor de Ciências Biológicas of the Federal University of Paraná, established by the DECREE Nº 787/03-BL on June 11th 2003, based upon the RESOLUTION Nº 01/03-BL from May 9th 2003, and upon the CEUA internal regiment, CERTIFIES that the procedures using animals in the research project specified below are in agreement with the ethical principals established by the Experimental Animal Brazilian Council (COBEA), and with the requirements of the "Guide for the Care and Use of Experimental Animals (Canadian Council on Animal Care)".

PROCESSO: 23075.032141/2013-46 **APROVADO**: 25/09/2013 – R.O. 08/2013

TÍTULO: Efeito quercetina em modelos animais de mania

AUTORES: Roberto Andreatini, Inara Fernanda M. Raupp-Barcaro, Manuela Lucas de melo, Paulo Sérgio Beirão Junior

DEPARTAMENTO: Farmacologia

Prof. Dr. Aleksander Roberto Zampronio
Coordenador da CEUA