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Dissertação apresentada ao Curso de Mestrado do programa em Ciências Farmacêuticas Universidade Federal de Alfenas, como requisito parcial para a obtenção do título de Mestre em Ciências Farmacêuticas. Área de concentração: Obtenção de insumos farmacêuticos e avaliação da atividade biológica.

Orientador: Prof. Dr. Alexandre Giusti-Paiva

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ABSTRACT

Sonchus oleraceus has been used to relieve pain in Brazilian folk medicine and in culinary. Nevertheless, available scientific information regarding this species is scarce; there are no reports related to its possible effect on the central nervous system. This study evaluated the antinociceptive, anxyolitic and antidepressant-like of hydroethanolic and dichloromethane extracts of *S. oleraceus* .The formalin, hot plate, and tail immersion tests as well as acetic acid-induced writhing were used to investigate the antinociceptive activity in mice. The anxyolitic effect of *S. oleraceus*i was evaluated n mice submitted to the elevated plus-maze and open-field tests. The putative antidepressant-like effects of extracts was evaluated on the performance of male mice in the forced swimming test (FST) and tail suspension test (TST) models predictive of antidepressive drugs. The extracts at test doses of 30-300 mg/kg, p.o. clearly demonstrated antinociceptive activity in formalin, hot plate, tail immersion and acetic acid-induced writhing tests. The extracts administered at 300 mg/kg, p.o. had a stronger antinociceptive effect than indomethacin (5 mg/kg, p.o.) and morphine (10 mg/kg, p.o.). In the elevated plus-maze test, the *S. oleraceus* extracts increased the percentage of open arm entries and time spent in the open-arm portions of the maze. The extracts induce an anti-thigmotactic effect, evidenced by increased locomotor activity into the central part of the open field set-up. The extracts administered at 30- 300 mg/kg, p.o. had a similar anxiolytic effect to clonazepam (0.5 mg/kg, p.o.). The immobility time in both FST and TST was significantly reduced by acute oral treatment with the extracts (dose range 100–300 mg/kg), without accompanying changes in ambulation, as assessed in an open-field test. This excluded the possibility that the effect of the extracts is due to an activation of locomotion. The efficacy of the extracts was found to be comparable to that of amitriptyline (10 mg/kg, p.o.). The extracts of *Sonchus oleraceus* markedly demonstrated antinociceptive, anxyolitic and antidepressant-like action in mice.

Key-words: *Sonchus oleraceus,* antinociceptive, anxyolitic and antidepressant.

RESUMO

Sonchus oleraceus é usada na medicina popular brasileira para aliviar dores em geral e na culinária. Entretanto, faltam informações científicas sobre esta espécie e não há relatos de seu possível efeito no sistema nervoso central. Este estudo avaliou os efeitos antinociceptivo, ansiolítico e antidepressivo dos extratos hidroetanólico e diclorometânico de *S. oleraceus*. Os testes da formalina, placa quente, imersão da cauda e contorções induzidas por ácido acético foram usados para investigar a atividade antinociceptiva em camundongos. O efeito ansiolítico de *S. oleraceus* foi avaliado em camundongos submetidos aos testes labirinto em cruz elevado e campo aberto. O efeito antidepressivo dos extratos foi avaliado no desempenho de camundongos machos no nado forçado e no teste de suspensão pela cauda que são modelos preditivos de fármacos antidepressivos. Os extratos nas doses de 30-300 mg/kg, v.o. demonstraram atividade antinociceptiva nos testes da formalina, placa quente, imersão da cauda e contorções induzidas por ácido acético. Os extratos administrados na dose de 300 mg/kg, v.o. tiveram um efeito maior que a indometacina (5 mg/kg, v.o.) e a morfina (10 mg/kg, v.o.). No teste do labirinto em cruz elevado, os extratos de *S. oleraceus* aumentaram a porcentagem de entradas e tempo nos braços abertos. Os extratos induziram um efeito anti-tigmotático evidenciado por um aumento da atividade locomotora dos animais na parte central do campo aberto. Os extratos administrados nas doses de 30-300 mg/kg, v.o. exerceram um efeito ansiolítico similar ao clonazepam (0,5 mg/kg, v.o.). O tempo de imobilidade em ambos os testes nado forçado e suspensão pela cauda foi reduzido significativamente com a administração dos extratos nas doses 100–300 mg/kg, v.o., sem mudanças na atividade locomotora, como foi mostrado pelo teste do campo aberto. Isso exclui a possibilidade de que o efeito do extrato possa ser devido a uma ativação locomotora. A eficácia dos extratos foi comparada a amitriptilina (10 mg/kg, v.o.). Os extratos de *Sonchus oleraceus* demonstram atividade antinociceptiva, ansiolítica e antidepressiva em camundongos.

Palavras-chave: *Sonchus oleraceus*, antinociceptivo, ansiolítico e antidepressivo.

SUMÁRIO

1 REVISÃO DE LITERATURA

1.1 PLANTAS MEDICINAIS

O uso de plantas no tratamento e na cura de enfermidades é tão antigo quanto à espécie humana. E ainda nos dias de hoje, nas regiões mais pobres do país e até mesmo nas grandes cidades brasileiras, plantas medicinais são comercializadas em feiras livres, mercados populares e encontradas em quintais residenciais (MACIEL et al., 2002).

As plantas medicinais são freqüentemente utilizadas com o intuito de substituir ou auxiliar as terapias convencionais no tratamento de várias doenças. Entre outros fatores, a preferência na utilização das plantas medicinais decorre da facilidade de obtenção e do baixo custo. Porém, sabe-se que as plantas medicinais apresentam ampla diversidade de metabólitos secundários com diferentes atividades biológicas (FARNSWORTH et al., 1985; SIMÕES, 2003), justificando a necessidade de um aprofundamento no conhecimento das propriedades dos produtos naturais derivados de vegetais e sua utilização na formulação de medicamentos. O maior problema para sua utilização terapêutica no tratamento convencional das diversas doenças é a falta de dados científicos que comprovem a eficácia e a segurança dos medicamentos preparados a partir das plantas medicinais.

Ainda que os medicamentos derivados de plantas tenham uma boa aceitação pela população e estejam presentes no mercado farmacêutico, apenas uma pequena parcela das plantas medicinais possui dados científicos que comprovem sua eficácia e seu espectro toxicológico, assim como garantia de qualidade do produto. Considerando-se os diversos metabólitos secundários presentes nas plantas, as principais categorias de princípios ativos derivados de plantas são os terpenóides, glicosídeos, alcalóides e outros tipos. Como exemplos relevantes, pode-se mencionar a morfina (*Papaver somniferum*), a digoxina (*Digitalis sp.*), o taxol (*Taxus brevifolia*), o quinino (casca da *Chinchona sp*), a vincristina e a vinblastina (alcalóides de *Catharanthus roseus*) (RATES, 2001).

Outras plantas como *Hypericum perforatum* que possui a hipericina, *Ginkgo biloba* que contém cerca de 24% de flavonóides e *Valeriana officinalis* que possui mais de 100 constituintes (não sendo conhecido os responsáveis por seu efeito sedativo), são exemplos da diversidade de espécies utilizadas para fins medicinais fornecendo substrato para a produção

de compostos biologicamente ativos ou compostos passíveis de modificações e otimizações estruturais que dão origem às entidades químicas (SCHULTZ, 1998). E ainda, *Panax ginseng* conhecido como ginseng, é uma das espécies medicinais mais famosas e preciosas consumida em todo o mundo (TYLER, 1995). Embora exiba múltiplas atividades biológicas, os mecanismos de seus efeitos ainda são desconhecidos. Recentemente evidências demonstraram que ginsenosídeos, o principal componente do ginseng e derivado de triterpeno, produzem sua atividade farmacológica por modular proteínas de membrana tais como canais de íon voltagem-dependente (KIM et al., 2002; LEE et al., 2007; NAH et al., 1995; SALA et al., 2002).

O uso popular de plantas medicinais contribui de forma significativa para a obtenção de informações terapêuticas importantes, que foram sendo acumuladas durante séculos. De maneira indireta, este tipo de cultura medicinal despertou o interesse de pesquisadores em estudos envolvendo áreas multidisciplinares sobre a flora mundial (MACIEL et al., 2002), em especial a flora brasileira (PINTO et al., 2002).

1.2 FAMÍLIA ASTERACEAE

Asteraceae é também conhecida por Compositae e muitas de suas espécies são usadas no cultivo devido ao seu valor biológico. É uma família que possui distribuição cosmopolita, sendo a maior família de Eucotiledôneas, com aproximadamente 1600 gêneros e 23000 espécies. No Brasil a família está bem representada, ocorrendo aproximadamente 300 gêneros e 2000 espécies (CONCEIÇÃO, 1982; LORENZI, 2000).

As espécies da família Asteraceae são amplamente distribuídas pelo mundo, mas é particularmente abundante no oeste dos Estados Unidos e do México, no sul do Brazil, ao longo dos Andes, nas áreas Mediterrâneas, Sudeste Asiático, Ásia Central, Sul da África e Austrália (BREMER, 1994).

Muitas Asteraceae são cultivadas como ornamentais, podendo ser destacadas a margarida (*Leucanthemum vulgare*) e os crisântemos (*Chrysanthemum* ssp). Pertecem a esta mesma família o girassol (*Helianthus annuus*), a alface (*Lactuca sativa*), a chicória, o almeirão e a escarola (as três pertencentes a *Cichorium intybus*). Diversas plantas medicinais estão também incluídas entre as Asteraceae, destacando-se a carqueja (*Baccharis sp*), o guaco

(*Mikani*a ssp), a mil-folhas (*Achillea millefolium*), a losna (*Artemisia absinthium)*, a serrelha *(Sonchus oleraceus)* e outras (SOUZA; LORENZI, 2005).

Plantas dessa família são extensivamente estudadas quanto a sua composição química e atividade biológica, sendo que algumas têm proporcionado o desenvolvimento de novos fármacos, inseticidas, entre outros. Inúmeros trabalhos científicos realizados com espécies da família Asteraceae apresentaram o isolamento de uma variedade de metabólitos secundários com destaque aos terpenos, flavonóides e poliacetilenos, que são responsáveis por diversas atividades biológicas (DAVINO, 1989).

1.2.1 *Sonchus oleraceus*

A espécie *Sonchus oleraceus* conhecida popularmente por serralha, pertence a família das Asteraceae e é encontrada em quase todo o mundo sendo originária da Europa e Norte da África (CUNHA, 1989; VIEIRA; BARRETO, 2000).

É uma planta anual, herbácea, leitosa, ereta, pouco ramificada, com reprodução por sementes. Possui um sabor amargo e paladar que lembra o espinafre. É usada em saladas e também utilizada com fins medicinais. Cresce espontâneamente em solos agrícolas de quase todo o país, onde é considerada planta daninha (VIEIRA; BARRETO, 2000).

S. oleraceus é geralmente encontrada próxima às cercas e muros nos quintais e nos terrenos baldios alcançando entre 40-110 cm de altura. Suas folhas são levemente serreadas e esta espécie possui flores amarelas como demosntrado na figura abaixo.

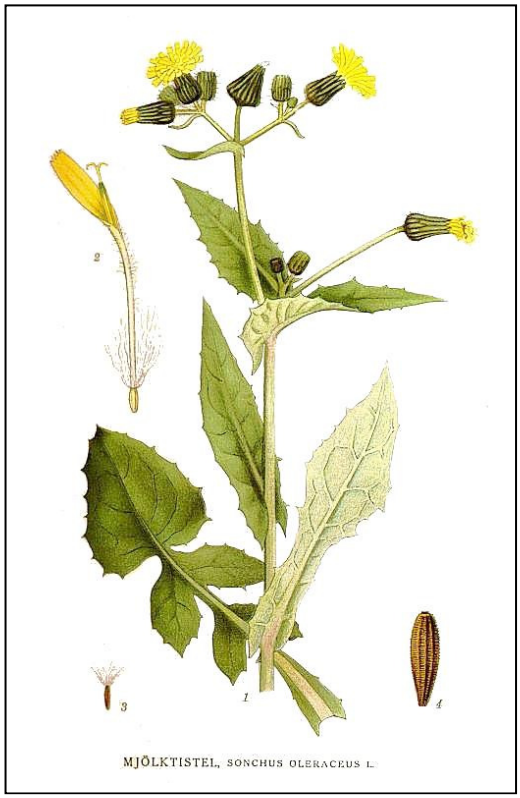


Figura 01: Partes aéreas de *Sonchus oleraceus*

Na medicina tradicional brasileira, as partes aéreas de *S.oleraceus* são usadas em saladas, infusão ou decocção e é administrada oralmente para o tratamento de dores estomacal, hepatite, infeccçoes, inflamação, dores de cabeça e de dente, dores em geral e reumatismo (DUARTE et al., 2002; VENDRUSCOLO; AGRA et al., 2007).

Na Itália, *S. oleraceus* é usada como depurativo, laxante e para facilitar a função hepática e intestinal (MANGANELI; TOMEI, 1999; GUARRERA, 2003). No Paquistão, as raízes e folhas são usadas como diurética, laxativa e tônica (GHAZANFAR, 1994).

A planta contém triterpenos pentacíclicos como taraxasterol (Figura 02) o qual teve um efeito analgésico comprovado e flavonóides como apigenina 7-glicuronida (antioxidante) e luteonina 7-glicosídeo (Figura 03) (ESPINOSA et al., 2008). Alcalóides, cumarinas, flavonóides e saponinas foram detectados nessa espécie tendo alguns destes divesras e importantes atividades biológicas (MIYASE; FUKUSHIMA, 1987; GHAZANFAR, 1994; GUARRERA et al., 2008).

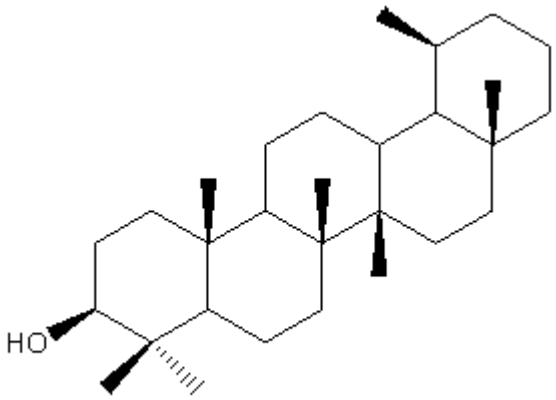


Figura 02: Estrutura química do taraxasterol isolado de *S. oleraceus*

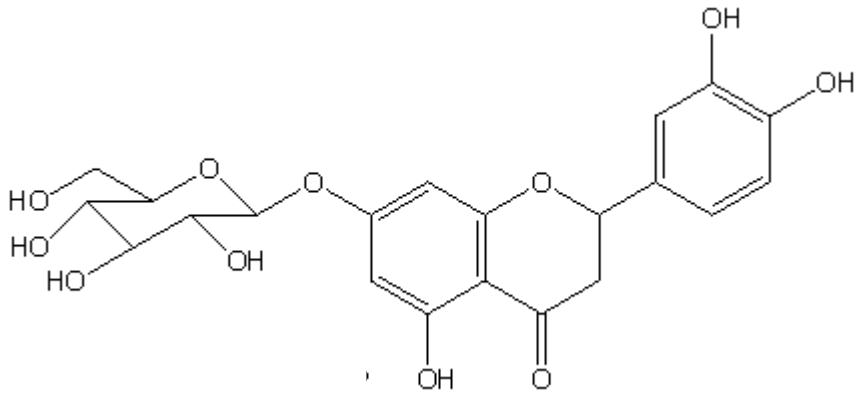


Figura 03: Estrutura química da luteonina 7-glicosídeo isolada de *S. oleraceus*

Além disso, há relatos de propriedade antioxidante do extrato de *S. oleraceus* sendo que flavona (luteolina), flavonóides (quercetrina e isoquercitina) e derivados de glicosídeos isolados mostraram-se responsáveis por esta propriedade (SCHAFFER et al., 2005; YIN et al., 2008).

Um estudo dos constituintes químicos de *S. oleraceus* coletada no Japão revelou a presença de seis compostos que foram isolados e elucidados sendo todos triterpenos. São eles: lupeol (Figura 04) que possui atividade antiinflamatória, α-amirina e β-amirina (Figura 05) que demonstraram possuir efeitos analgésicos, ácido ursônico (Figura 06), ácido oleanólico e ácido betulínico (Figura 06) possuindo esses três últimos atividade antiinflamatória (NGUEMFO et al., 2009; MESSIAS et al., 2008; BAI et al., 2008). Outros estudos ainda

revelaram a presença de acetato de germanicil (XU; LIANG, 2005). Entretanto nenhum teste biológico foi realizado com esses compostos isolados da espécie *S. oleraceus*, sendo que as atividades atribuídas são de compostos pertencentes a outras espécies.

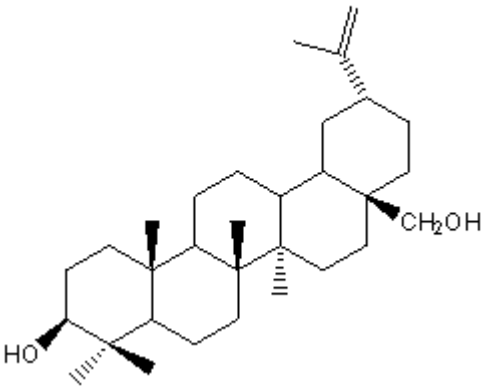
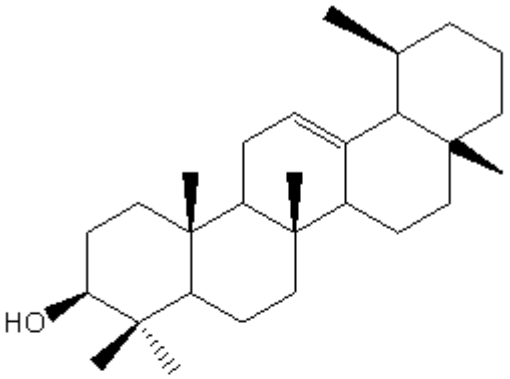
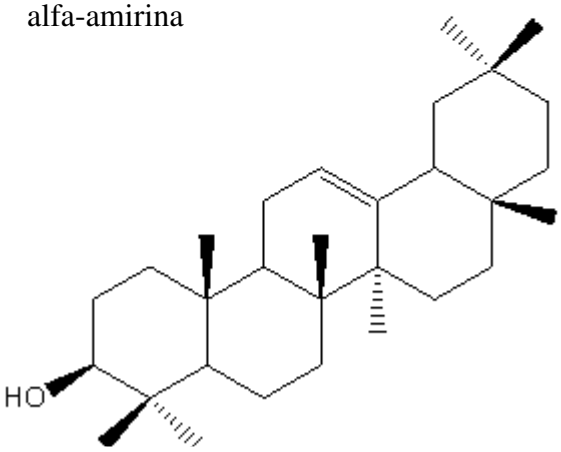


Figura 04: Estrutura química do lupeol isolado de *S. oleraceus*

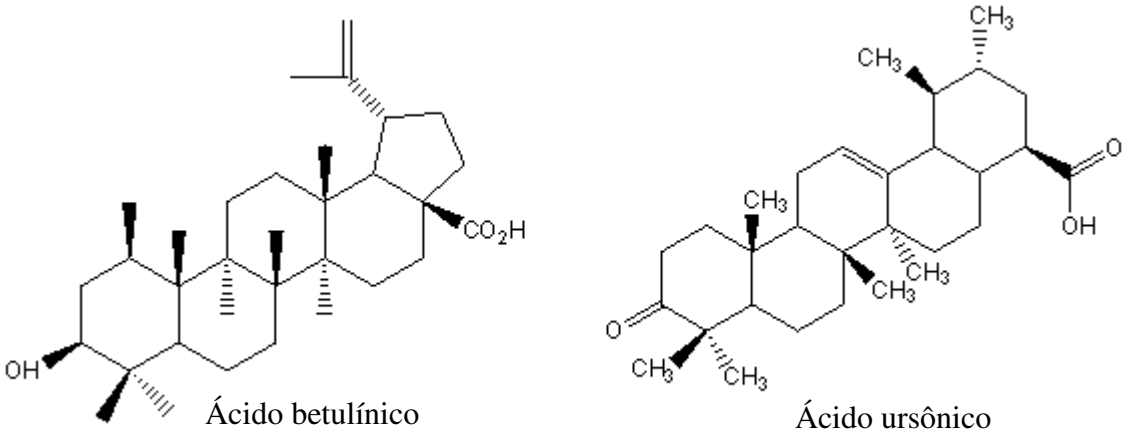


alfa-amirina



beta-amirina

Figura 05: Estrutura química de amirinas isoladas de *S. oleraceus*



Ácido betulínico Ácido ursônico

Figura 06: Estrutura química do ácido betulínico e ácido ursônico isolados de *S. oleraceus*

A espécie S. *oleraceus* é conhecida popularmente por possuir diversas atividades como analgésica e "fortificante dos nervos" as quais nos fazem pensar que ela possa agir no SNC. Porém o maior problema para sua utilização terapêutica no tratamento convencional das diversas doenças é a falta de dados científicos que comprovem a eficácia e a segurança dos preparados a partir dessa planta. Para isso, são utilizados modelos em animais a fim de verificar um possível efeito antinociceptico, ansiolítico e analgésico.

1.3 DOR E NOCICEPÇÃO

1.3.1 Definições

A dor é uma experiência complexa e de componentes afetivo-motivacionais e descriminativo, que geralmente origina-se no sítio da lesão, sendo transmitida pelo sistema nervoso periférico, processada em diversos níveis do sistema nervoso central e, finalmente, percebida no córtex cerebral (PRADO; DEL BEL, 1998). Pode ser definida, segundo o Comitê de Taxonomia da Associação Internacional para o Estudo da Dor (I.A.S.P.), como uma sensação ou experiência desagradável associada com um dano tecidual real ou potencial, ou descrita como tal dano (MERSKEY; BOGDUK, 1994; MILLAN, 1999).

É um mecanismo de demarcação de limites para o organismo e de aviso sobre a ocorrência de estímulos lesivos provenientes do meio externo ou do próprio organismo. A importância dessa função protetora exigiu da natureza o desenvolvimento de todo um sistema sensorial próprio para veicunlar as informações nociceptivas (LENT, 2004).

Nocicepção é um termo aplicado aos mecanismos neurológicos que detectam o estímulo lesivo e é desprovido do componente afetivo-motivacional (FERREIRA, 2004). Essa detecção de lesão tecidual ocorre através de transdutores especializados ligados a fibras dos nervos periféricos do tipo A delta e fibras do tipo C. Se a estimulação dos nociceptores vai resultar ou não em dor depende de inúmeros fatores moduladores. A intensidade da dor percebida varia consideravelmente, dependendo do humor do indivíduo, da quantidade de distração com relação à dor, e das sugestões positivas ou negativas de outras pessoas, assim como de vários processos neurológicos periféricos e centrais que são capazes de modular a transmissão nas sinapses nas vias nociceptivas (SCHAILDE; RICHTER, 2004).

Diante da simplicidade da sensação da dor e da complexidade em seu mecanismo, muitas pesquisas etnofarmacológicas tradicionais no uso de plantas medicinais para o alívio da dor são vistas como estratégia produtiva e lógica na procura por novas drogas analgésicas (ELISABETSKY et al., 1995).

Muitos compostos químicos presentes em diversas plantas possuem propriedades analgésicas (CALIXTO et al., 2000). Exemplos disso, os alcalóides presentes em *Papaver somniferum*, os canabinóides da *Cannabis sativa*, a salicina e o ácido salicílico presente em *Salix spp* e inúmeros alcalóides, terpenóides, esteróides, flavonóides, xantonas, taninos, lactonas, glicosídeos (HUA et al., 1997; CALIXTO et al., 2000).

1.3.2 Mecanismos da dor

Os receptores para dor (nociceptores) são terminações nervosas livres de fibras mielínicas delgadas Aδ e fibras nervosas amielínicas finas C que se distribuem por praticamente todos os tecidos do organismo (superfície cutânea, parede das vísceras ocas, parênquima das vísceras sólidas, vasculatura, ossos e articulações, córnea, raízes dentárias) com exceção do SNC (LENT, 2004).

Uma vez estimulados química, mecânica ou termicamente, os nociceptores produzem potencias receptores como todos os demais receptores sensoriais, e esses são codificados em potenciais de ação na membrana vizinha à extremidade especializada na transdução. Após essas estimulações, a informação nociceptiva é transmitida da periferia para as áreas talâmicas e corticais por dois grandes sistemas de fibras ascendentes: o sistema anterolateral e o sistema trigemial. O primeiro é responsável pela transmissão nociceptiva da região de troncos e membros, e o segundo, associa-se à percepção dolorosa da região da face (MARQUEZ, 2004).

A informação nociceptiva gerada pela despolarização no nociceptor é conduzida pelas fibras aferentes primárias tipo C não-mielinizadas e por fibras tipo Aδ levemente mielinizadas (neurônios de primeira ordem) e retransmitidas para neurônios de projeção (neurônios de segunda ordem) localizados no corno dorsal da medula espinhal e do trigêmio. Chegando à medula espinhal, as fibras aferentes são principalmente organizadas nos feixes ascendentes, principalmente o neoespinotalâmico (sensibilidade tipo discriminativa, neurossensorial) e o palioespinotalâmico (dimensão afetiva). Essas vias terminam no córtex sensorial e límbico, respectivamente, após sinapses no tálamo (BEAR et al, 2002).

Fibras tipo Aδ e C levam informações ao sistema nervoso central (SNC) em diferentes velocidades devido às diferenças nas velocidades de condução de seus potenciais de ação.

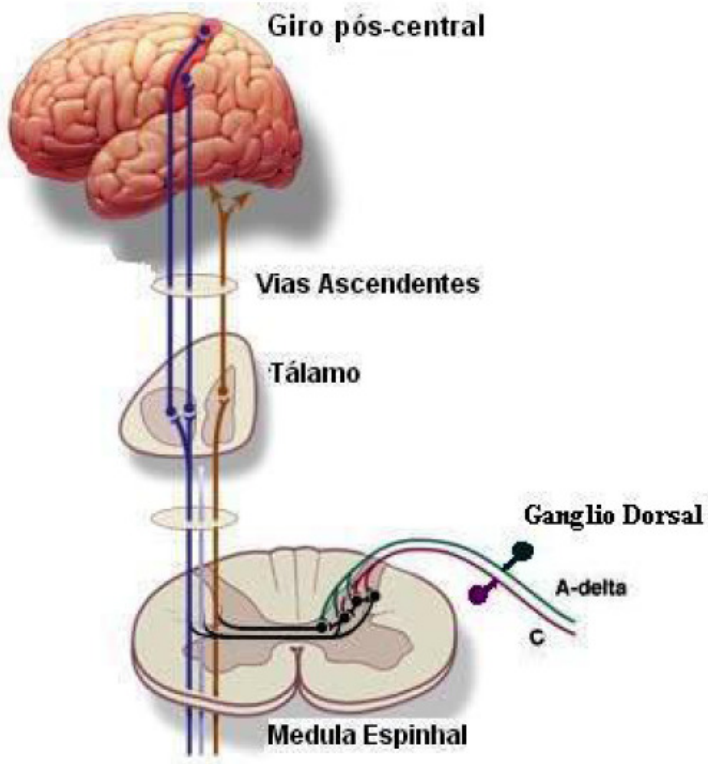


Figura 07: Representação esquemática dos neurônios da via ascendente

O principal neurotransmissor liberado pelas fibras Aδ e C é o glutamato, o qual gera um rápido potencial de ação excitatório nos neurônios de segunda ordem. As terminações nervosas das fibras do tipo C também liberam na fenda sináptica vários neuropeptídeos, dentre eles a substância P que desencadeará um lento potencial de ação excitatório nos neurônios de segunda ordem. Há, portanto uma ação coordenada do glutamato e dos neuropeptídeos para que ocorra a transmissão da informação nociceptiva (LENT, 2004).

1.3.2.1 Vias descendentes envolvidas na modulação da dor

Sabe-se que muitas classes de neurônios da coluna dorsal, os próprios neurônios aferentes primários e fibras descendentes do cérebro exercem um grande potencial modulatório sobre a transferência da informação nociceptiva. Após entrar no corno dorsal da medula espinhal, a informação nociceptiva proveniente das vísceras está sujeita ao

processamento por uma diversidade de mecanismos, alguns dos quais potencializando e outros inibindo sua tranferência para centros supra-espinhais (MILLAN, 2002).

As células do corno posterior podem ser inibidas pela transmissão de informações nociceptivas aos centros superiores por algumas regiões cerebrais como, o córtex cerebral, a formação reticular da medula oblonga e a substância cinzenta periaquedutal. As fibras descendentes dessas áreas inibem a transmissão das fibras aferentes nociceptivas aos neurônios de projeção, atuando sobre os interneurônios inibidores do corno posterior (COHEN, 2001).

O sistema analgésico endógeno é um mecanismo que interfere no limiar da dor. Ele age nas estruturas do corno posterior da medula, através de neurotransmissores (serotonina, noradrenalina, endorfina), que são liberados no tronco cerebral, levando a uma modulação do estimulo álgico (LENT, 2004).

O fenômeno doloroso sofre um mecanismo de modulação na substância gelatinosa da medula e no tálamo, que é explicada pela teoria das comportas de Melzack e Wall. As fibras Aδ, mielínicas, de condução rápida, responsáveis pela condução de estímulos táteis, dolorosos e pressão, agem aumentando a ativação de interneurônios da substância gelatinosa e fechando a comporta. As fibras C são fibras amielínicas, de condução lenta e que conduzem estímulos dolorosos. Essas fibras agem de forma inversa as fibras Aδ, ocasionando a abertura da comporta, transmitindo o efeito álgico. Os estímulos provenientes do cérebro, dependendo da interpretação dada aos estímulos aferentes, podem abrir ou fechar as comportas. Desta forma, gera um mecanismo segmentar e um supra-segmentar de inibição ou falta de inibição do fenômeno doloroso (MARQUEZ, 2004; BROOKS ; TRACEY, 2005).

Muitas espécies vegetais atuam inibindo ou diminuindo a dor, como são o caso de anestésicos que agem nas vias ascendentes, ou analgésicos como os opióides (morfina) que são agonistas dos receptores opióides e importantes na regulação normal da sensação da dor ou inibindo processos inflamatórios.

O processo inflamatório é um mecanismo benéfico e fisiológico pelo qual o organismo se defende contra infecções e tenta reparar danos teciduais ou perda de função (LAWRENCE et al., 2002). A palavra inflamação é derivada do “estado de se estar inflamado”. Inflamar significa “colocar fogo” o que implica na cor vermelha, na possibilidade de aquecimento e na geração de dor (TROWBRIGDE; EMLING, 1996).

1.3.3 Avaliação experimental da resposta nociceptiva

Vários modelos “in vivo” são utilizados na pesquisa de compostos com atividade analgésica como o teste da formalina, que consiste de uma injeção subcutânea de formalina em ratos ou camundongos determinando o aparecimento de uma gama de respostas motoras bem caracterizadas, cuja quantificação permite que se avalie a intensidade da resposta nociceptiva (DUBUISSON; DENNIS, 1977). A primeira fase, nos primeiros cinco minutos após a injeção da formalina, é atribuída um caráter neurogênico, sendo sensível a analgésicos opióides e a alguns agonistas das vias descendentes. A segunda fase 15-30 minutos após a injeção é mais bem caracterizada como dor de origem inflamatória, sendo sensível a analgésicos antiinflamatórios não esteroidais (DICKENSON; SULLIVAN, 1987; YAKSH et al., 2001).

O teste de imersão da cauda permite a obtenção de informações adicionais sobre o mecanismo e o local da atividade antinociceptiva detectada, uma vez que o parâmetro avaliado – reflexo de retirada da cauda – é de integração medular (IRWIN et al., 1951). Um aumento no tempo de reação do animal ao estímulo é geralmente considerado um importante parâmetro para avaliar a atividade antinociceptiva central (RUJJANAWATE et al., 2003). Este teste é capaz de diferenciar analgésicos periféricos e centrais (ASONGALEM et al., 2004).

Teste da placa quente é outro tipo de teste que permite verificar a atividade antinociceptiva de compostos, no caso dos extratos de *S. oleraceus.* O animal sobre uma superfície aquecida a 50ºC determina uma resposta característica, na qual o animal troca rapidamente o apoio dos pés (“sapateia”), levanta ou lambe uma das patas. A latência para o aparecimento dessa resposta, cronometrado em segundos, pode ser considerada como indicativo da intensidade da resposta nociceptiva a um estímulo térmico (WOOLFE; MACDONALD, 1944; BARS, 2001).

Já o teste de contorções adominais induzidas por ácido acético caracteriza-se por contração e rotação do abdômen, seguida pela extensão de uma ou ambas as patas traseiras. Esta resposta motora decorre da aplicação de um estímulo nociceptivo por via intraperitoneal. A contagem do número de contorções ocorridas em um intervalo de tempo pré-determinado é tomada como índice da resposta nociceptiva tempo (KOSTER et al., 1959).

Com a finalidade de descartar possíveis efeitos sobre a atividade locomotora, o teste do campo-aberto pode ser usado para avaliar a atividade exploratória dos animais, ou seja, o número de cruzamentos com as quatro patas entre as divisões do campo na periferia, verificando se os extratos realmente tiveram atividade antinociceptiva ou se a dor causada pelos estímulos citados anteriormente foi diminuída ou inibida devido a um decréscimo da atividade locomotora indicativa de um possível relaxamento muscular (MONTGOMERY, 1955).

1.4 ANSIEDADE

A ansiedade é uma desordem psiquiátirca de etiologia complexa e ainda não entendida completamente. Juntamente com o medo constituem as primeiras respostas naturais de defesa frente a um perigo potencial. O medo que sentimos pode ser rápido e passageiro ou mais lento e duradouro. Nos dois casos, tudo depende da natureza do estímulo que o provoca. Às vezes estamos distraídos e um ruído subido e intenso nos provoca um susto, que desaparece quando percebemos que o estímulo é inócuo e já cessou. Outras vezes o estímulo é realmente ameaçador, e permanece prolongando o medo. Há situações ainda que o estímulo seja virtual: não está necessariamente presente, embora possa acontecer a qualquer momento. Nesse caso o medo se prolonga ainda mais. Quando isso ocorre continuamente durante muito tempo o sentimento se transforma em um estado de estresse ou tensão, e chega a um estado de emoção chamada ansiedade (LENT, 2004; KIM; GORMAN, 2005).

Quando um ainmal é confrontado com uma ameaça à sua integridade física ou à própria sobrevivência, ele apresenta um conjunto de respostas comportamentais e neurovegetativas que caracterizam a reação do medo. Pelo menos no homem, sabemos que estas respostas vêm acompanhadas de uma experiência extremamente desagradável (GRAEFF, 2005).

A ansiedade é a emoção semelhante ao medo. Porém, enquanto este é o fruto de ameaça definida, na ansiedade a fonte de perigo é incerta ou desconhecida. Contudo, as alterações psicofisiológicas que compõem a ansiedade são semelhantes às do medo, podendo-se admitir a identidade básica dos mecanismos neurais que integram ambos os estados emocionais (BLANCHARD; BLANCHARD, 1988; GUIMARÃES; GRAEFF, 2001).

A ansiedade e o medo produzem mudanças fisiológicas por meio da ativação do sistema simpático que reflete em aumento de batimentos e da força de contração do coração. Esses efeitos são percebidos como palpitações, tremores, sudorese, sensação de falta de ar acompanhada de hiperventilação ou parada respiratória. Pode ocorrer também hipersecreção gástrica, aumento da motilidade intestinal e urgência para micção devido à ativação da divisão parassimpática do sistema neurovegetativo (GUIMARÃES; GRAEFF, 2001).

Na ansiedade os ajustes fisiológicos extrapolam o âmbito do sistema nervoso autônomo e atingem o sistema endócrino e imunitário. A ativação da divisão simpática causa também a estimulação da glândula adrenal, cujas células secretam adrenalina e noradrenalina (NA) que mimetizam as ações da divisão simpática do sistema nervoso autônomo. A liberação desses hormônios na corrente sanguínea acentua e prolonga as manifestações fisiológicas como taquicardia, sudorese e piloereção. Além disso, sob ativação contínua da amígdala (“botão disparador acionado pelos estímulos causadores do medo”) e por retroação da concentração sanquínea aumentada de drenalina e noradrenalina, o hipotálamo passa a secretar hormônios liberadores do hormônio adrenocorticotrófico (ACTH). Este hormônio ativará a córtex adrenal, provocando a secreção sistêmica de hormônios glicocorticóides, que têm efeitos sobre o metabolismo da maioria das células do organismo. Porém os mesmos podem ter também ação anti-imunitária e anti-inflamatória podendo provocar queda da resistência às infecções (LENT, 2003; VAN de KAR; BLAIR, 1999).

Os benzodiazepínicos é a classe mais importante usada para tratar os estados de ansiedade. Atuam de modo seletivo sobre os receptores A do GABA que mediam a transmissão sináptica inibitória rápida, através do SNC. Os benzodiazepínicos potencializam a resposta ao GABA, por facilitarem a abertura dos canais de cloreto ativados pelo GABA (KATZUNG, 2006). Os principais efeitos desses fármacos são: redução da ansiedade e da agressão, sedação e indução do sono, redução do tônus musular e da coordenação, e possuem ainda efeito anticonvulsivante (ARGYROPOULOS; NUTT, 1999).

Outros fármacos são usados para amenizar os efeitos da ansiedade, entre eles pode-se citar: a buspirona que é um agonista parcial dos receptores 5-HT e possui atividade ansiolítica com pequena sedação, e os barbitúricos que são depressores não-seletivos do SNC e produzem efeitos que vão da sedação e redução da ansiedade à inconsciência e morte por falência respiratória e cardiovascular. Os inibidoresde recaptação de 5-HT, como a fluoxetina e inibidores de captação mistos 5-HT/NA, são usados como fámacos antidepressivos e também mostram eficáica nos distúrbios da ansiedade.

Entretando, os fármacos ansiolíticos produzem muitos efeitos indesejáveis como aminésia, dependência, síndrome de abstinência, reação paradoxal e decréscimo da função psicomotora (LADER; MORTON, 1991; KAN et al., 1997; SCHWEIZER; RICKELS, 1998). Diante disso, tem-se a necessidade de novos fármacos ansiolíticos com menor potencial de induzir reações adversas.

Muitas plantas utilizadas pela medicina popular são capazes de atuar no comportamento, humor e sensações, e o entendimento de seus mecanismos de ação, segurança e eficácia, é um desafio para os pesquisadores. Entre essas plantas pode-se citar *Passiflora incarnata*, *Valeriana officinalis* e *Piper methysticum* (CARLINI, 2003). Na procura de novas substâncias ansiolíticas e em virtude de que muitas plantas atuam no SNC, foram utilizados modelos em animais a fim de verificar o efeito ansiolítico dos extratos da planta em estudo.

1.4.1 Avaliação experimental da resposta ansiolítica

Além de um componente subjetivo (emocional) da ansiedade humana, há efeitos comportamentais e fisiológicos mensuráveis, que também ocorrem em animais de experimentção. Esses efeitos podem ser observados nos seguintes testes como no teste do campo-aberto que é usado para avaliar a atividade ansiolítica dos animais considerando o número de cruzamentos, com as quatro patas, no centro. Um aumento desse parâmetro indica uma possível atividade ansiolítica ou sedativa. Tipicamente os animais tendem a ficar por mais tempo na periferia em comparação com a área central. Essa preferência é conhecida como tigmotaxia e o inverso leva a um efeito anti-tigmotático que pode ser obervado no campo aberto e pode ser referido como uma ação ansiolítica (VALLE, 1970).

O teste do labirinto consiste em um aparato com dois braços abertos, dois braços fechados e uma plataforma central. Os animais são colocados no cento de frente para um dos braços fechados e filmados por cinco minutos. As medidas comportamentais registradas são: freqüência de entradas e o tempo despendido nos braços abertos e nos fechados. Um aumento seletivo nos parâmetros correspondentes aos braços abertos (entradas e tempo) revela um efeito ansiolítico, uma vez que caracterísiticas aversivas aos braços abertos são consideradas suficientes para produzir um comportamento padrão exibidas pelos animais (PELLOW et al., 1985; LISTER, 1987; FILE et al., 1990, CAROBREZ; BERTOGLIO, 2005).

1.5 DEPRESSÃO

A depressão representa um problema mundial de saúde pública devido a sua prevalência e ao impacto fisiológico. Pessoas de diferentes regiões do mundo têm utilizado plantas medicinais para aliviar as desordens afetivas. Esse tipo de terapia pode ser uma alternativa no tratamento de depressão e uma fonte para a farmacoterapia de doenças psíquicas (ZHANG, 2004; SARRIS, 2007).

Muitas espécies vegetais possuem efeito antidepressivo em modelos animais e já foram elucidados seus mecanismos de ação como *Apocynum venetum* que possui flavonóides atuando no sistema monoaminérgico e *Hypericum perforatum* que contém flavonóides, hiperforina, hipericina e rutina agindo por inibição de recaptação de 5-HT e NA e inibição da MAO (CAO et al., 2003; CERVO et al., 2000; DAUDT et al., 2000; NOLDNER; SCHOTZ, 2000; GAMBARANA et al., 2001).

Diversos fatores participam da gênese das depressões, entre estes se podem citar os neurofisiológicos. Tais fatores continuam sendo alvo de pesquisas, uma vez que, ainda não se encontram totalmente elucidados. A principal teoria bioquímica da depressão é que esta é causada por um déficit funcional das monoaminas (MAO) (como serotonina e norepinefrina) transmissoras em certos locais do cérebro, ou de seus receptores ineficientes, enquanto a mania resultada de um excesso funcional (LAFER; FILHO, 1999). A função dopaminérgica reduzida também está implicada na fisiopatologia da depressão (RAMPELLO et al., 2000).

As principais drogas usadas no tratamento da depressão são agentes tricíclicos inibidores de recaptação de NA e 5-HT. Os compostos tricíclicos inibidores de recaptação de NA e 5-HT bloqueiam a recaptação de NA e 5-HT pela membrana neuronal do terminal pré-sináptico, aumentando sua concentração na fenda sináptica. Outros compostos como inibidores da MAO levam a um acúmulo do neurotransmissor nas vesículas sinápticas, aumentando assim a quantidade de norepinefrina liberada por impulso nervoso. (NARANJO, 2001; NASH; HACK, 2002).

A depressão pode ter várias causas, incluindo predisposição genética e familiar, distúrbios clínicos e psiquiátricos e uso de alguns fármacos. Seu tratamento é amplo uma vez que a depressão constitui como uma síndrome complexa de gravidade variada.

1.5.1 Avaliação experimental da resposta antidepressiva

A Farmacologia experimental usa conceitos e técnicas derivados tanto da Farmacologia clássica quanto da Psicologia, para estudar as interações entre drogas e comportamentos. Alguns estudos investigam processos comportamentais ou psicológicos que são alterados pela administração de drogas. Entre esses estudos, estão os testes "in vivo" que são utilizados na pesquisa de compostos com atividade antidepressiva.

O teste do campo aberto permite uma avaliação da atividade estimulante dos animais sendo que o número de cruzamentos, com as quatro patas, entre as divisões do campo na periferia aumentado pode indicar que a substância que foi administrada anteriormente, exerceu atividade estimulante e não antidepressiva (MONTGOMERY, 1955).

Outro teste usado na investigação da atividade antidepressiva é o teste da suspensão pela cauda, onde os camundongos são pré-tratados com os diferentes compostos e submetidos ao teste proposto por Stéru et al. (1985). O procedimento experimental consiste em suspender os animais pela cauda, por um período de seis minutos, no qual é registrado o tempo total de imobilidade para cada animal a partir do segundo minuto. Uma diminuição no tempo de imobilidade do animal relaciona-se com um efeito antidepressivo.

E por fim, o teste do nado forçado é um modelo de indução de estresse utilizado nesse estudo. Esse teste é feito de acordo com o método descrito por Porsolt e colaboradores (1997). É utilizado um cilindro vertical de vidro, com dimensões de 14 cm de diâmetro e 25 cm de altura, preenchido com água à 30ºC até a altura de 20 cm. O volume de água deve permitir que o animal possa nadar ou boiar ("float") sem encostar as patas ou a cauda no fundo do recipiente. Para o teste, cada camundongo é colocado no cilindro por seis minutos e será avaliado o tempo total boiando (tempo em que o animal faz pequemos movimentos somente para manter a cabeça acima do nível da água) e somente os quatro últimos minutos são analisados. Como no teste de suspensão pela cauda, o tempo de imobilidade diminuído indica um efeito antidepressivo.

Muitas hipóteses têm sido desenvolvidas para explicar a adaptação física que é a imobilidade observada nos dois últimos testes citados acima (CRYAN; MOMBEREAU, 2004). Uma das hipóteses e a mais aceita é baseada na idéia de que o animal "perde a esperança de escapar" de tal situação, em outras palavras a falta de persistência em escapar é percibida como uma desistência e refletida em tempo de imobilidade descrito como um estado

depressivo (THYERRY et al.,1984). Substâncias antidepressivas revertem esse quadro diminuindo assim o tempo de imobilidade fazendo com que o animal não desista de escapar das cituaçoes impostas a ele.

REFERÊNCIAS BIBLIOGRÁFICAS

AGRA, M. F. et al., Medicinal and poisonous diversity of the flora of "Cariri Paraibano", Brazil. *Journal of Ethnopharmacology* 111, 383-395. 2007.

ARGYROPOULOS, S. V.; NUTT, D. J. The use of benzodiazepines in anxiety and other disorders. *Eur. Neuropsychopharmacol.* 9 Suppl. 6, p. S407–S412. 1999.

ASONGALEM, E. A. et al., Antiinflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. *Journal of Ethnopharmacology* 95, 63–68. 2004.

BAI, Y. et al., Chemical constituents of Japanese herb *Sonchus oleraceus*. *Journal of China Pharmaceutical University*, v.39. p 279-281, 2008.

BARS, D. L. et al., Animal Models of Nociception. *Pharmacol Rev* 53: 597-652, 2001.

BEAR, M. et al., *Neurociências: desvendando o sistema nervoso*. Tradução de Jorge Alberto Quillfeldt. 2. ed. Porto Alegre: Artmed, 2002.

BLANCHARD, D. C.; BLANCHARD, R. J. Ethoexperimental approaches to the biology of emotion. *Annu Rev Psychol* 39. P. 43-68. 1988.

BREMER, K. *Asteraceae and Classification.* Timber Press, p. 13, 1994.

BROOKS, J.; TRACEY, I. From nociception to pain perception: imaging the spinal and supraspinal pathways. *Journal of Anatomy***,** n.207, p.19-33, 2005.

CALIXTO, J. B. et al., Naturally occurring antinociceptive substances from plants. *Phytother. Res.* 14 (2000), pp. 401–418. 2000.

CAO, Y. et al., Determination of hydroxyl radical by capillary electrophoresis and studies on hdroxyl radical scavenging activities of Chinese herbs. *Analytical and Bioanalytical Chemistry* 376, p. 691–695. 2003.

CARLINI, E. A. Plants and the central nervous system. *Pharmacology Biochemistry and Behaviour,* v. 75. p 501-512, 2003.

CAROBREZ, A. P.; BERTOGLIO, L. J. Ethological and temporal analyses of anxiety-like behavior: The elevated pluz-maze model 20 years on. *Neuroscience and Biobehavioral Reviews,* v. 29 .p 1193-1205. 2005.

CERVO, L. et al., Role of hyperforin in the antidepressant-like activity of *Hypericum perforatum* extracts. *Psychopharmacology (Berl)* 164, p. 423–428. 2002.

COHEN, H. *Neurociência para fisioterapeutas: incluindo correlações clinicas*. 2. ed. São Paulo: Manole, 2001.

CONCEIÇÃO. M. *As Plantas Medicinais no ano de 2000.* Tao Editora Ltda. São Paulo-SP, 2 ed., 152p. 1982.

CRYAN, J. F.; MOMBEREAU, C. In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry* 9, 326-357. 2004.

CUNHA, R. W. *Estudo Fitoquímico e Ensaios Biológicos de Lichnophora rupestris, Samir Leitão (Vernoniae, compositae).* Tese (Doutorado em Ciências): Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, USP. 160f. 1989.

DAUDT, R. et al., Screening for the antidepressant activity of some species of *Hypericum* from South Brazil. *Phytotherapy Research* 14, p. 344–346. 2000.

DAVINO, S. C. *Estudo in vitro da atividade anitifúngica e antibacteriana de extrato de plantas brasileiras da família Compositae (Asteraceae) e alguns de seus constituintes.* Dissertação (Mestrado em Ciências). USP. São Paulo, 115f, 1989.

DICKENSON, A. H.; SULLIVAN, A. F., Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurons. *Neuroscience Letters* 83, 207–211. 1987.

DUARTE, M. G. et al., Phytochemical screening and in vitro antibacterial activity of weed plants. *Lecta* 20, 177-182. 2002.

DUBUISSON, D., DENNIS, S.G. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain 4*, 161–174. 1977.

ELISABETSKY. E. et al., Analgesic activity of *Psychotria colorata* (Willd. ex R. & S.) Muell. Arg. alkaloids. *Journal of Ethnopharmacology***.** 48:77-83, 1995.

ESPINOSA, P. F. et al., Antinociceptive, hypoglycemic and spasmolytic effects of Brickellia veronicifolia. *Journal of Ethnopharmacology* 118(3), p. 448-454. 2008.

FARNSWORTH, N. R. The development of pharmacological and chemical research for application to traditional medicine in developing countries. *Journal of Ethnopharmacology,* v.2, p.173-181, 1980.

FERREIRA, J. *Participação de receptores B1 e B2 para as cininas em modelos experimentais de dor crônica*, Tese de Doutorado em Farmacologia. Universidade Federal de Santa Catarina, 2004.

FILE P. S. et al., Characterization of phenomenon of 'one-trial tolerance' to the anxiolytic effect of chlordiazepoxide in the elevated plus-maze, *Psychopharmacology* 102. p. 98–101. 1990.

GAMBARANA, C. et al., A study of the antidepressant activity of *Hypericum perforatum* on animal models. *Pharmacopsychiatry* 34, p. S42–S44. 2001.

GHAZANFAR, S. A., Handbook of Arabian medicinal plants. *CRC Press*. 1994.

GRAEFF, F. G. *Drogas psicóticas e seu modo de ação***.** 2. ed. Revista e ampliada. São Paulo: EPU, 2005.

GUIMARÃES, F. S.; GRAEFF, F. G. *Fundamentos de psicofarmacologia***.** Atheneu, p123-159, 2001.

GUARRERA, P. M. et al., Ethnophytotherapeutical research in the high Molise region (Central-Southern Italy). *Journal of Ethnobiology and Ethnomedicine* 4, 7. 2008.

GUARRERA, P. M. Food medicine and minor nourishment in the folk traditions of Central Italy (Marche, Abruzzo and Latium). *Fitoterapia* 74, 515-544. 2003.

HUO, B. S., QIN, M. J. Content analysis of flavonoids in Five species of *Sonchus* L. *Journal of Plant Resources and Environment*. v. 17, p.77-78. 2008.

IRWIN, S. et al., The effect of morphine, methadone and meperidine on some reflex of spinal animais to nociceptive information. *J Pharmacol Exp Ther* 101: 132-143. 1951.
Janeiro. 9 ed. 2006.

KAN, C. C. et al., High prevalence of benzodiazepine dependence in out-patient users, based on the DSM-III-R and ICD-10 criteria. *Acta Psychiatr. Scand.* 96, p. 85–93. 1997.

KATZUNG, B. G. *Farmacologia Básica e Clínica*. Editora Guanabara Koogan S.A. Rio de

KIM, J., GORMAN, J. The pshychobiology of anxiety. *Clinical Neuroscience Research.* V.4, p. 335-347, 2005.

KIM, S. et al., Inhibitory effect of ginsenosides on NMDA receptor-mediated signals in rat hippocampal neurons. *Biochem Biophys Res Commun 296*:247-254. 2002.

KOSTER, R. et al., Acetic acid for analgesic screening. *Fed Proc* 18: 412-421. 1959.

LADER, M; MORTON, S. Benzodiazepine problems. *Br. J. Addict.* 86, p. 823–828. 1991.

LAFER, B.; FILHO, H. P. V. *Genética e fisiopatologia dos transtornos depressivos.* Brás. Psiquiatria, Depressão, v. 21, 1999.

LAWRENCE, T. et al., Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nat Rev Immunol* 2(10):787-95. 2002.

LEE, B. H. et al., Identification of ginsenoside interaction sites in 5-HT(3A) receptors. *Neuropharmacology 52*:1139-1150. 2007.

LENT, R. *Cem Bilhões de Neurônios:* Conceitos Fundamentais de Neurociência, São Paulo: Editora Atheneu, 2004.

LISTER, R. G. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92, 180–185. 1987.

LORENZI, H. *Plantas Daninhas do Brasil-terrestres, aquáticas, parasitas e tóxicas.* 3 ed., Instituto Plantarum. Nova Odessa, SP. 2000.

MACIEL, M. A. M. et al., Plantas medicinais: a necessidade de estudos multidisciplinares *Quimica Nova,* v.25, n.3, p.429-438, 2002

MANGANELLI, R. E.; TOMEI, P. E. Ethnopharmacobotanical studies of the Tuscan Archipelago. *Journal of Ethnopharmacology* 65, 181-202. 1999.

MARQUEZ, J. O. *Dor, diagnóstico e tratamento: Bases de anatomia e fisiopatologia*. v.1, n.1, 2004.

MERSKEY, H.; BOGDUK, N. Classification of chronic pain: description of chronic pain

MESSIAS, K. L. S. et al., Chemical composition and analgesic activity of the leaves and branches of Marlierea tomentosa Camb. *Química Nova* 31(7), p. 1747-1749**.** 2008.

MILLAN, M. J. Descending control of pain. *Progress in Neurobiology*, V. 66, p. 355-374, 2002.

MILLAN, M. J. The induction of pain: an integrative review. *Progress in Neurobiology,* v.57,n.1, p.164, 1999.

MIYASE, T.; FUKUSHIMA, S. Studies on sesquiterpene glycosides from *Sonchus oleraceus*. *Chemical and Pharmaceutical Bulletin* 35, 2869-2874. 1987

MONTGOMERY, K. C. The relation between fear induced by novel stimulation and exploratory behavior. *J. Comp. Physiol. Psychol* 48: 254-260. 1955.

NAH, S. Y. et al., A trace component of ginseng that inhibit Ca2+ channels through a pertussis toxin-sensitive G protein. *Proc Natl Acad Sci USA 92*:8739-8743. 1995.

NARANJO, C. A. et al., The role of the brain reward system in depression. *Prog Neuropsychopharmacol Biol Psychiatry* 25(4):781-823. 2001.

NASH, L. T.; HACK, S. The pharmacological treatment of anxiety disorders in children and adolescents. *Expert Opin. Pharmacother* 3: 555-571. 2002.

NGUEMFO, E. L. et al., Anti-oxidative and anti-inflammatory activities of some isolated constituents from the stem bark of Allanblackia monticola Staner L.C (Guttiferae). *Inflammopharmacology* 17 (1), p. 37-41. 2009.

NOLDNER, M.; SCHOTZ, K. Rutin is essential for the antidepressant activity of *Hypericum perforatum* extracts in the forced swimming test. *Planta Medica* 68, p. 577–580. 2002.

PELLOW, S. et al., Validation of open, closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neurosciences Methods* 14 1, 49–167. 1985.

PINTO, A. C. et al., Produtos naturais: atualidade, desafios e perspectivas. *Química Nova,* São Paulo, v.25, n.1, p.45-61, jan./fev. 2002.

PORSOLT, R. D. et al., Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266: 730–732. 1977.

PRADO, P. T. C.; DEL BEL, E. A. C-*fos*, an immediate early gene as a neuromaker for nociception. *Medicina, Ribeirao Preto,* v.31, p.424-433, 1998.

RAMPELLO, L. et al., Dopamine and depression. Therapeutic implications. *CNS Drugs*, v. 13, p. 36-45, 2000.

RATES, S. M. K. *Plants as sources of drugs.* Toxicon, Oxford 39:603-13, 2001.

RUJJANAWATE, C. et al. Pharmacological effect and toxicity of alkaloids from *Gelsemium elegans* Benth. *Journal of Ethnopharmacology* 89, 91–95. 2003.

SALA, F. et al., Effects of ginsenoside Rg2 on human neuronal nicotinic acetylcholine receptors. *J Pharmacol Exp Ther* 301:1052-1059. 2002.

SARRIS, J. Herbal medicines in the treatment of psychiatric disorders: a systematic review.

SCHAFFER, S. Antioxidant properties of Mediterranean food plant extracts: geographical differences. *Journal of Physiology and Pharmacology* 56 Suppl 1, 115-124. 2005.

SCHAILDE, H. G; RICHTER, F. Pathophysiology of pain. *Current concepts in clinical surgery.* 389: 237-243. 2004.

SCHULTZ, V. Rational Phytotherapy: *A Physician's Guide to Herbal Medicine*. Berlin, Springer-Verlag, 1998.

SCHWEIZER, E.; RICKELS, K. Benzodiazepine dependence and withdrawal: a review of the syndrome and its clinical management. *Acta Psychiatr. Scand.* 98 Suppl. 393, pp. 95–101. 1998.

SHARIFIFAR, F. et al., Major flavonoids with antioxidant activity from Teucrium polium L. *Food Chemistry* 112(4), p. 885-888. 2009.

SIMÕES, C. M. O. *Farmacognosia: da planta ao medicamento*. Porto Alegre-RS, Editora da UFSC e UFRGS. 3. ed, 2003.

SOUZA, V. C.; LORENZI, H. *Botânica Sistemática***.** Instituto Plantarum. Nova Odessa, SP. 2005.

STERU, L. et al., The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* 85: 367-370. 1985
syndromes and definitions of pain terms. *IASP Press,* Seattle, 1994.

THIERRY, B. et al., Searching-wainting strategy: a candidate for an evolutionary model of depression? *Behav Neural Biol* 41. 180-189. 1984.

TROWBRIGDE, H. O.; EMLING, R. C.; *Mediadores químicos da resposta vascular*. In: Inflamação uma revisão do processo. Quitessence Publishing Co. Inc., São Paulo, 27-42, 172. 1996.

TYLER, V. E. Herbal remedies. *J Pharm Technol 11*:214-220. 1995.

VALLE. F. P. Effects of stain, sex and illumination on open-field behavior of rats. *Am J Psychol* 83: 103-111. 1970.

VAN de KAR, L. D; BLAIR, M. L. Forebrain pathways mediating stress-induced hormone secretion. *Front Neuroendocrinol* 20*,* p. 1-48, 1999.

VENDRUSCOLO, G. S., MENTZ, A.Z. Ethnobotanical survey of the medicinal plants used by the community of Ponta Grossa neighborhood, Porto Alegre, Rio Grande do Sul, Brazil. *Iherigia Série Botânica* 61; 83-103. 2006.

VIEIRA, B. S., BARRETO, R. W. First record of Bremia lactucae infecting *Sonchus oleraceus* and *Sonchus asper* in Brazil and its infectivity to lettuce. *Journal of Phytopathology* 154, 84-87. 2000.

XU, Y., LIANG, J.Y. Chemical constituents of *Sonchus oleraceus* L. *Journal of China Pharmaceutical University*, v. 36, 411-413. 2005.

WOOLFE. G.; MACDONALD, A. L. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). *J. Pharmacol Exp Ther* 80: 300-307. 1944.

YAKSH, T. L. et al., An automated flinch detecting system for use in the formalin nociceptive bioassay. *Journal of Applied Physiology* 90, 2386–2402. 2001.

YIN, J., et al. Antioxidant activity of flavonoids and their glucosides from *Sonchus oleraceus* L. *Journal of Applied Biological Chemistry*. v. 51, p. 57-60. 2008.

ZHANG, Z. J. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. *Life Sci*;75:1659-1699. 2004.

2 MATERIAL E MÉTODOS – RESULTADOS E DISCUSSÃO

Como descrito nas normas do Programa de Pós-graduação em Ciências Farmacêuticas, a critério do orientador e do discente, a dissertação pode ser apresentada sob a forma de 01 (um) volume contendo: uma revisão de literatura seguida de artigos científicos referentes aos resultados obtidos no desenvolvimento da pesquisa. Para tanto foram redigidos três artigos, todos já submetidos.

2.1 ARTIGO I

O artigo I mostra os materias e métodos detalhados e os resultados do efeito antinociceptivo dos extratos hidroetanólico e diclorometânico das partes aéreas de *Sonchus oleraceus* em modelos animais.

O artigo foi submetido na revista Journal of Ethnopharmacology com fator de impacto 2.049, em 26 de setembro de 2008.

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Alfenas–MG, September 26th, 2008

R. Verpoorte
Editor-in-Chief, Journal of Ethnopharmacology

Dear Dr. Verpoorte;

We would like to submit the manuscript entitled "**Evaluation of the antinociceptive activity of extracts of *Sonchus oleraceus* in mice**" to be considered for publication in the Journal of Ethnopharmacology.

On the basis of the traditional claim that *S. oleraceus* and the lack of scientific studies to establish its potential pharmacological properties, the objective of this study was to evaluate the antinociceptive effect of *S. oleraceus* extract in mice. All experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of Alfenas.

The manuscript, or part of it, has not been, and will not be submitted elsewhere for publication. All authors listed consented to the submission, and all data are used with the consent of the individual responsible for generating the data.

Sincerely,

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Evaluation of the antinociceptive activity of extracts of *Sonchus oleraceus* in mice

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Abstract

Ethnopharmacological relevance: *Sonchus oleraceus* has been used to relieve pain in Brazilian folk medicine.

Aim of the study: This study was conducted to establish the antinociceptive properties of hydroethanolic and dichloromethane extracts from aerial parts of *Sonchus oleraceus*.

Materials and methods: The formalin, hot plate, and tail immersion tests as well as acetic acid-induced writhing were used to investigate the antinociceptive activity in mice.

Results: The extracts at test doses of 30-300 mg/kg, p.o. clearly demonstrated antinociceptive activity in all tests. The extracts administered at 300 mg/kg, p.o. had a stronger antinociceptive effect than indomethacin (5 mg/kg, p.o.) and morphine (10 mg/kg, p.o.).

Conclusion: The extracts of *Sonchus oleraceus* markedly demonstrated antinociceptive action in mice, which supports previous claims of its traditional use.

Key words: Nociception; Inflammatory pain; Analgesia; Tail flick assay; Tail formalin test; Writhing test

1. Introduction

Sonchus oleraceus L. (Asteraceae) has a widespread world distribution and is also common in Brazil. It is considered to be native to Europe and North Africa and is commonly problematic as an invader of many crops (Vieira and Barreto, 2000).

S. oleraceus is edible to humans as a leaf vegetable and is frequently consumed in Mediterranean countries (Guil-Guerrero et al., 1998; Guarrera, 2003; Heinrich et al., 2005; Guarrera et al., 2006; Lentini and Venza, 2007), Australia (Liu et al., 2007) and New Zealand, particularly by the native Maori (Cambie and Ferguson, 2003). In South of Minas Gerais (Southeast of Brazil), *S. oleraceus* is very important in the diets of rural people, especially seasonally, and provides cash income to the harvester and relatively inexpensive vegetables to the community.

In Brazilian traditional medicine, aerial parts of *S. oleraceus*, popularly known as "serralha," are used mostly in salad, infusion or decoction, and is administered orally for treating stomachic pain, hepatitis, infections, inflammation, headaches, general pain, rheumatism and toothaches (Duarte et al., 2002; Vendruscolo and Mentz, 2006, Agra et al., 2007). In Italy, *S. oleraceus* is used as a depurative and laxative and to facilitate hepatic and intestinal function (Manganeli and Tomei, 1999, Guarrera, 2003). In Pakistan, the roots and leaves are used as a febrifuge, diuretic, laxative and general tonic (Ghazanfar, 1994). The plant contains taraxasterol, apigenin 7-glucuronide, and luteolin 7-glucoside (Ghazanfar, 1994). Alkaloids, coumarins, flavonoids and saponins have also been detected (Miyase and Fukushima, 1987; Ghazanfar, 1994; Guarrera et al., 2008). In addition, antioxidant properties of the *S. oleraceus* extract have previously been reported (El and Karakaya, 2004; Schaffer et al., 2005).

Based on the traditional claims surrounding *S. oleraceus* and the lack of scientific studies on its potential pharmacological properties, the objective of this study was to evaluate the antinociceptive properties of extracted aerial parts of *S. oleraceus* in mice.

2. Material and methods

2.1. Plant material

Aerial parts of *S. oleraceus* (Asteraceae) were collected in March 2008 in Alfenas, Minas Gerais, Brazil. Dr. Geraldo A. da Silva, Department of Pharmacy of Federal University of Alfenas, identified the plant, and the voucher specimen is deposited at the Herbarium of Federal University of Alfenas–MG (voucher number 0194).

2.2. Preparation of the plant extracts and reference drugs

Aerial parts of *S. oleraceus* were air-dried at 40ºC and powdered. For the hydroethanolic extract (SoHE), the dried powder was extracted by percolation with 50% ethanol at room temperature. The solvent was removed under reduced pressure and then dried with a spray dryer (BÜCHI Mini Spray Dryer B-290). For the dichloromethanic extract (SoDE), the dried powder was extracted by percolation with dichloromethane at room temperature, and the solvent was removed under reduced pressure. The residues were used for bioactivity determination.

S. oleraceus extracts (SoHE and SoDE) were administered in 30, 100, and 300 mg/kg doses after being suspended in vehicle (1% sodium carboxymethylcellulose suspension in distilled water). Indomethacin (5 mg/kg) and morphine sulphate (10 mg/kg) in vehicle were used as reference drugs. Test drugs were orally administered, except for morphine sulphate, which was intraperitoneally administered in an equivalent volume of 10 ml/kg body weight of the animal.

2.3. Assessment of antinociceptive activity of *S. oleraceus* extracts

Adult male Swiss mice (22–28 g), obtained from the Central Animal Facility of Federal University of Alfenas, were housed in a temperature-controlled room with access to water and

food ad libitum until they were used. All experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of Federal University of Alfenas.

2.3.1. Acetic acid-induced writhing in mice

Acetic acid (0.6% v/v, 10 ml/kg) was injected into the peritoneal cavities of mice, which were placed in a large glass cylinder, and the intensity of nociceptive behavior was quantified by counting the total number of writhes occurring between 0 and 20 min after stimulus injection, as described earlier (Collier et al. 1968). Oral treatments (p.o.) with vehicle, indomethacin, SoHE or SoDE were given one hour prior to acetic acid injection (n = 6 per group). Morphine sulphate was intraperitoneally administered (i.p) 30 min before the test. The writhing response consists of a contraction of the abdominal muscle together with a stretching of the hind limbs. The antinociceptive activity was expressed as writhing scores over a period of 20 min.

2.3.2. Formalin-induced nociception

A formalin solution (5% in 0.9% saline; 20µl/paw) was injected into the hind paw plantar surface (i.pl.), and the animals were individually placed in transparent observation chambers, as previously described (Santos and Calixto, 1997). Oral treatments (p.o.) with vehicle, indomethacin, SoHE or SoDE were given 1 h prior to formalin injection (n = 8 per group). Morphine sulphate was administrated (i.p) 30 min before the test. The time spent in licking the injected paw was recorded and expressed as the total licking time in the early phase (0–5 min) and late phase (20–30 min) after formalin injection.

2.3.3. Tail immersion test

The lower two-thirds of the tail was immersed in a beaker containing water kept at 50 ± 0.5°C (Wang et al., 2000). The time in seconds until the tail was withdrawn from the water was defined as the reaction time. The reaction time was then measured 0, 30, 60, and 120 min after the oral administration of vehicle, SoHE, SoDE and morphine (n = 8 per group), with the reaction time of zero minutes being the start of the test. The mice were exposed to hot water for no longer than 20 s to avoid tissue injury.

2.3.4. Hot plate test

The hot plate was an electrically heated surface kept at a constant temperature of 50.0 ± 0.5°C. Mice were placed on the heated surface within the Plexiglas walls to constrain their locomotion on the plate, and the latency to a discomfort reaction (licking of the paws or jumping) was recorded 0, 30, 60, and 120 min after oral administration of vehicle, SoHE, SoDE or morphine (n = 8 per group), with the reaction time of zero minutes being the start of the test. A cut-off time of 20 s was chosen to indicate complete analgesia and to avoid tissue injury. The latencies for paw licking or jumping were recorded for each animal.

2.3.5. Open-field test

In order to discard the possible nonspecific muscle relaxants or the sedative effects of extract, the motor performance of the mice was evaluated on the open-field apparatus (Archer, 1973). Groups of mice (n = 6) were treated with vehicle (10 ml/kg, p.o.), SoHE or SoDE (30, 100 and 300 mg/kg, p.o.) one hour before the performance of the test. Each animal was placed in the center of the open-field arena and allowed to have free ambulation for 5 min of observation of the locomotion frequency (number of floor units the animal entered on all its limbs).

2.4. Evaluation of acute toxicity of the *S. oleraceus* extracts

SoHE or SoDE was orally administered to a group of mice, both male and female. The behavior parameters observed after administration were convulsion, hyperactivity, sedation, grooming, and increased or decreased respiration during a period of seven days. Food and water were provided ad libitum.

2.5. Statistical analysis

The data obtained were analyzed using the GraphPad software program Version 4.0 and expressed as a mean ± S.E.M. Statistically significant differences between groups were calculated by the application of an analysis of variance (ANOVA) followed by the Newman-Keuls test. P–values less than 0.05 ($p < 0.05$) were used as the significance level.

3. Results

3.1. Acetic acid-induced writhing in mice

The oral administration of SoHE (30-300mg/kg) and SoDE (100 and 300 mg/kg) caused a significant reduction in the number of writhing episodes induced by acetic acid compared to the control. Indomethacin and morphine produced a 61.1% and 98.2% reduction in acetic acid-induced writhing movements compared to the control. The results are provided in Fig. 1.

3.2. Formalin test in mice

The SoHE and SoDE at doses of 30-300mg/kg, p.o. had a significant antinociceptive activity compared to the control in both the early and late phases. The reference drug, indomethacin, suppressed only the second phase of the formalin test, while morphine inhibited both phases of the pain stimulus (Fig. 2).

3.3. Tail immersion and hot plate tests induced nociception in mice

As demonstrated in Fig. 3, SoHE (Fig. 3A) and SoDE (Fig. 3B) administrated at doses of 30–300 mg/kg caused a significant increase in the tail-flick response latency time as compared to the control animals. In the hot plate test, oral treatment with SoHE (Fig. 4A) and SoDH (Fig. 4B) at doses of 30–300 mg/kg increased the latency time as compared to the control group. Morphine significantly increased the latency time in both tests.

3.4. Open-field test

Mice treated with SoHE or SoDE at 30–300 mg/kg did not cause a reduction in the numbers of crossings and rearings when compared to the control group in the open-field test (data not shown).

3.5. Acute toxicity

The SoHE or SoDE at a dose of 0.5–5 g/kg, p.o. given to mice had no affect on their behavioral responses during the observation period of seven days after administration. No mortality was observed up to seven days of monitoring. The LD_{50} value of these extracts in mice was therefore estimated to more than 5 g/kg, p.o. As the effective dose used in the present study (100 mg/kg, p.o.) was 50-fold less than the dose used in the acute toxicity test, it was safe to assume that the normal doses of 30, 100, and 300 mg/kg, p.o. given to mice in this study were safe.

4. Discussion

Sonchus oleraceus extracts demonstrated potent analgesic effects both in the visceral and central nociceptive mouse models. An intraperitoneal injection of acetic acid can produce peritoneal inflammation (acute peritonitis), which causes a response consisting of the contraction of the abdominal muscles accompanied by an extension of the forelimbs and elongation of the body. This writhing response is considered to be a visceral inflammatory pain model (Koster et al., 1959), and this method has been associated with increased levels of prostaglandins in the peritoneal fluids (Derardt et al., 1980). The results herein revealed that *S. oleraceus* extracts had significantly reduced acetic acid-induced writhing responses, which were similar to those of the reference drugs.

The antinociceptive effects of SoHE and SoDE were also verified on formalin-induced spontaneous nociceptive behaviors. An intraplantar injection of formalin is a commonly-used model of acute inflammatory pain, in which rodents display spontaneous nociceptive behaviors consisting of flinching/shaking of the affected hind paw in two distinct phases (Dubuisson and Dennis, 1977). The first and second phases are generally believed to reflect excitation of peripheral afferent nociceptors and central sensitization, respectively (Dickenson and Sullivan, 1987; Yaksh et al., 2001). Different mechanisms have been shown to be involved in first and second phase nociceptive behaviors, based on the differential pharmacology associated with these behaviors. For example, whereas second phase behaviors are selectively attenuated by cyclooxygenase inhibitors, first and second phase behaviors are attenuated by opioids (for review, see Yaksh et al., 2001). Like other substances that act on the central nervous system (CNS), *S. oleraceus* extracts inhibited both phases of the test in a manner similar to that of morphine. Moreover, the results of this test are in agreement with

those obtained in the hot plate and tail immersion tests, confirming the central antinociceptive effect of this extract.

In the tail immersion test, which consists of a thermal stimulus, an increase in the reaction time is generally considered to be an important parameter for evaluating central antinociceptive activity (Rujjanawate et al., 2003). This test is able to differentiate between central and peripheral analgesics (Asongalem et al., 2004). The antinociceptive activity of the *S. oleraceus* extracts was observed at a dose of 100 and 300 mg/kg, showing a similar effect to that of morphine. This test also revealed that the antinociceptive effect of *S. oleraceus* extracts on mice remained present for at least up to 120 min after administration of the extract. The tail-flick response is believed to be a spinally-mediated reflex (Chapman et al., 1985). The *S. oleraceus* extracts were found to have antinociceptive activity in the hot plate test, which is a specific central antinociceptive test. The antinociceptive effects of *S. oleraceus* extracts involve supraspinal as well as spinal components, as demonstrated by the use of the hot plate (Yaksh and Rudy, 1976; Yaksh and Rudy, 1977; Yeung et al., 1977) and tail immersion (Woolf et al., 1980; Luttinger, 1985) tests, respectively. The results suggest that *S. oleraceus* extracts have a central antinociceptive effect, as shown by the prolonged delay in response time when the mice were subjected to a nociceptive stimulus in the tail immersion test, and also by the increase in reaction time of the mice in the hot plate test.

The present study showed the efficacy of the *S. oleraceus* extracts in different antinociceptive responses generated by a chemical noxious stimulus produced by formalin or acetic acid injections or by a thermal noxious stimulus in the hot plate and tail immersion tests. The antinociceptive effects of *S. oleraceus* extracts occurred at doses that evoked no visible modification in the overall behavior of the animals. However, further studies should be carried out to investigate the molecular mechanism of action of the *S. oleraceus* extracts and their participation in the pain inhibitory mechanisms in the CNS.

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References

Agra, M.F., Baracho, G.S., Nurit, K., Basílio, I.J., Coelho, V.P., 2007. Medicinal and poisonous diversity of the flora of "Cariri Paraibano", Brazil. Journal of Ethnopharmacology 111, 383-395.

Archer, J., 1973. Tests for emotionality in rats and mice: a review. Animal Behavior 21, 205–235.

Asongalem, E.A., Foyet, H.S., Ekobo, S., Dimo, T., Kamtchouing, P., 2004. Antiinflammatory, lack of central analgesia and antipyretic properties of *Acanthus montanus* (Ness) T. Anderson. Journal of Ethnopharmacology 95, 63–68.

Cambie, R.C., Ferguson, L.R., 2003. Potential functional foods in the traditional Maori diet. Mutation Research 523-524, 109-117.

Chapman, C.R., Casey, K.L., Dubner, R., Foley, K.M., Gracely, R.H., Reading, A.E., 1985. Pain measurement: an overview. Pain 22, 1–31.

Collier, H.O., Dinneen, L.C., Johnson, C.A., Schneider, C., 1968. The abdominal constriction response and its suppression by analgesic drugs in the mouse. British Journal of Pharmacology and Chemotherapy 32, 295–310.

Deraedt, R., Jouquey, S., Delevallée, F., Flahaut, M., 1980. Release of prostaglandins E and F in an algogenic reaction and its inhibition. European Journal of Pharmacology 61, 17–24.

Dickenson, A.H., Sullivan, A.F., 1987. Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurons. Neuroscience Letters 83, 207–211.

Duarte, M.G., Soares, I.A., Brandão, M., Jacome, R.L., Ferreira, M.D., Silva, C.R., Oliveira, A.B., 2002. Phytochemical screening and in vitro antibacterial activity of weed plants. Lecta 20, 177-182.

Dubuisson, D., Dennis, S.G., 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4, 161–174.

El, S.N., Karakaya, S., 2004. Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. International Journal of Food Sciences and Nutrition 55, 67-74.

Ghazanfar, S.A., 1994. Handbook of Arabian medicinal plants. CRC Press.

Guarrera, P.M., 2003. Food medicine and minor nourishment in the folk traditions of Central Italy (Marche, Abruzzo and Latium). Fitoterapia 74, 515-544.

Guarrera, P.M., Lucchese, F., Medori, S., 2008. Ethnophytotherapeutical research in the high Molise region (Central-Southern Italy). Journal of Ethnobiology and Ethnomedicine 4, 7.

Guarrera, P.M., Salerno, G., Caneva, G., 2006. Food, flavouring and feed plant traditions in the Tyrrhenian sector of Basilicata, Italy. Journal of Ethnobiology and Ethnomedicine 2, 37.

Guil-Guerrero, J.L., Gimenez-Gimenez, A., Rodriguez-Garcia, I., Torija-Isasa, M.E., 1998. Nutritional composition of *Sonchus* species (*S asper* L, *S. oleraceus* L and *S. tenerrimus* L). Journal of the Science of Food and Agriculture 76, 628-632.

Heinrich, M., Leonti, M., Nebel, S., Peschel, W.,2005. "Local Food - Nutraceuticals": an example of a multidisciplinary research project on local knowledge. Journal of physiology and pharmacology 56 Suppl 1, 5-22.

Koster, R., Anderson, M., Beer, E.J., 1959. Acetic acid for analgesic screening. Federation Proceedings. 18, 412–416.

Lentini, F., Venza, F., 2007. Wild food plants of popular use in Sicily. Journal of Ethnobiology and Ethnomedicine 3, 15.

Liu, L., Howe, P., Zhou, Y.F., Hocart, C., Zhang, R., 2007. Fatty acid profiles of leaves of nine edible wild plants: an Australian study. Journal of Food Lipids 9, 65-71.

Luttinger, D., 1985. Determination of antinociceptive efficacy of drugs in mice using different water temperatures in a tail-immersion test. Journal of Pharmacogical Methods 13, 351–357.

Manganelli, R.E., Tomei, P.E., 1999. Ethnopharmacobotanical studies of the Tuscan Archipelago. Journal of Ethnopharmacology 65, 181-202.

Miyase, T., Fukushima, S., 1987. Studies on sesquiterpene glycosides from *Sonchus oleraceus*. Chemical and Pharmaceutical Bulletin 35, 2869-2874.

Rodrigues, E., Mendes, F.R., Negri, G., 2006. Plants indicated by Brazilian Indians for disturbances of the central nervous system: a bibliographical survey. Central Nervous System Agents in Medicinal Chemistry 6, 211-244.

Rujjanawate, C., Kanjanapothi, D., Panthong, A., 2003. Pharmacological effect and toxicity of alkaloids from *Gelsemium elegans* Benth. Journal of Ethnopharmacology 89, 91–95.

Santos, A.R., Calixto, J.B., 1997. Further evidence for the involvement of tachykinin receptor subtypes in formalin and capsaicin models of pain in mice. Neuropeptides 31, 381–389.

Schaffer, S., Schmitt-Schillig, S., Müller, W.E., Eckert, G.P., 2005. Antioxidant properties of Mediterranean food plant extracts: geographical differences. Journal of Physiology and Pharmacology 56 Suppl 1, 115-124.

Vendruscolo, G.S., Mentz, A.Z., 2006. Ethnobotanical survey of the medicinal plants used by the community of Ponta Grossa neighborhood, Porto Alegre, Rio Grande do Sul, Brazil. Iherigia Série Botânica 61; 83-103.

Vieira, B.S., Barreto, R.W., 2006. First record of Bremia lactucae infecting *Sonchus oleraceus* and *Sonchus asper* in Brazil and its infectivity to lettuce. Journal of Phytopathology 154, 84-87.

Wang, Y.X., Gao, D., Pettus, M., Phillips, C., Bowersox, S.S., 2000. Interactions of intrathecally administered ziconotide, a selective blocker of neuronal N-type voltage-sensitive calcium channels, with morphine on nociception in rats. Pain 84, 271–281.

Woolf, C.J., Mitchell, D., Barrett, G.D., 1980. Antinociceptive effect of peripheral segmental electrical stimulation in the rat. Pain 8, 237–252.

Yaksh, T.L., Ozaki, G., McCumber, D., Rathbun, M., Svensson, C., Malkmus, S., Yaksh, M.C., 2001. An automated flinch detecting system for use in the formalin nociceptive bioassay. Journal of Applied Physiology 90, 2386–2402.

Yaksh, T.L., Rudy, T.A., 1976. Analgesia mediated by a direct spinal action of narcotics. Science 192, 1357–1358.

Yaksh, T.L., Rudy, T.A., 1977. Studies on the direct spinal action of narcotics in the production of analgesia in the rat. The Journal of pharmacology and experimental therapeutics 202, 411–428.

Yeung, J.C., Yaksh, T.L., Rudy, T.A., 1977. Concurrent mapping of brain sites for sensitivity to the direct application of morphine and focal electrical stimulation in the production of antinociception in the rat. Pain 4, 23–40.

Figure 1. Effects of *S. oleraceus* hydroethanolic extract (SoHE) and dichloromethanic extract (SoDE) administered orally against acetic acid-induced writhing movements in mice. Animals were pretreated orally with vehicle, SoHE (doses 30, 100, and 300 mg/kg), SoHE (doses 30, 100, and 300 mg/kg), indomethacin (INDO; 5 mg/kg) or morphine (MORP; 10 mg/kg) prior to the acetic acid (0.6%, i.p.). Each column represents the mean with S.E.M. for six mice in each group. The asterisks denote the significance levels when compared with the control group (one-way ANOVA followed by Newman–Keuls test): *p<0.05; * p<0.01.

Figure 2. Effects of *S. oleraceus* hydroethanolic extract (SoHE) and dichloromethanic extract (SoDE) given by oral route on the licking induced by formalin in mice. Animals were pretreated orally with vehicle, SoHE (doses 30, 100, and 300 mg/kg), SoHE (doses 30, 100, and 300 mg/kg), indomethacin (INDO; 5 mg/kg) or morphine (MORP; 10 mg/kg) prior to formalin. The total time spent licking the hindpaw was measured in the first (Panel A) and second (Panel B) phases after intraplantar injection of formalin. Each column represents the mean with S.E.M. for eight mice in each group. The asterisks denote the significance levels when compared with the control group (one-way ANOVA followed by Newman–Keuls test): **p<0.01; ***p<0.001.

Figure 3. Effects of *S. oleraceus* hydroethanolic extract (SoHE, Panel A) and dichloromethanic extract (SoDE, Panel B) administered orally in the tail immersion test in mice. Animals were pretreated orally with vehicle, SoHE (doses 30, 100, and 300 mg/kg), SoHE (doses 30, 100, and 300 mg/kg) or morphine prior to the tail immersion test at 50°C. Each column represents the mean with S.E.M. for eight mice in each group. The asterisks denote the significance levels when compared with the control group (one-way ANOVA followed by Newman–Keuls test): *p<0.05; **p<0.01; ***p<0.001.

Figure 4. Effects of *S. oleraceus* hydroethanolic extract (SoHE, Panel A) and dichloromethanic extract (SoDE, Panel B) administered orally in the hot plate test in mice. Animals were pretreated orally with vehicle, SoHE (doses 30, 100, and 300 mg/kg), SoHE (doses 30, 100, and 300 mg/kg) or morphine prior to the hot plate test at 50°C. Each column represents the mean with S.E.M. for eight mice in each group. The asterisks denote the significance levels when compared with control group (one-way ANOVA followed by Newman–Keuls test): $*p<0.05$; $**p<0.01$; $***p<0.001$.

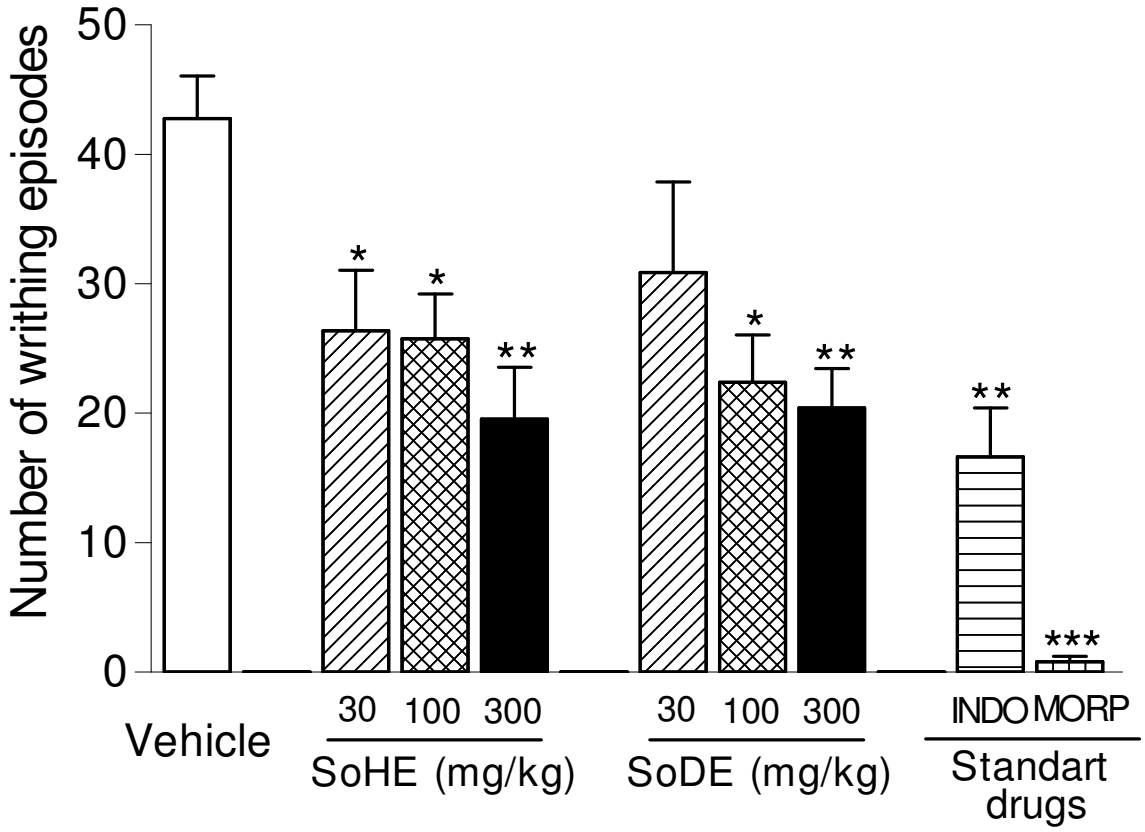
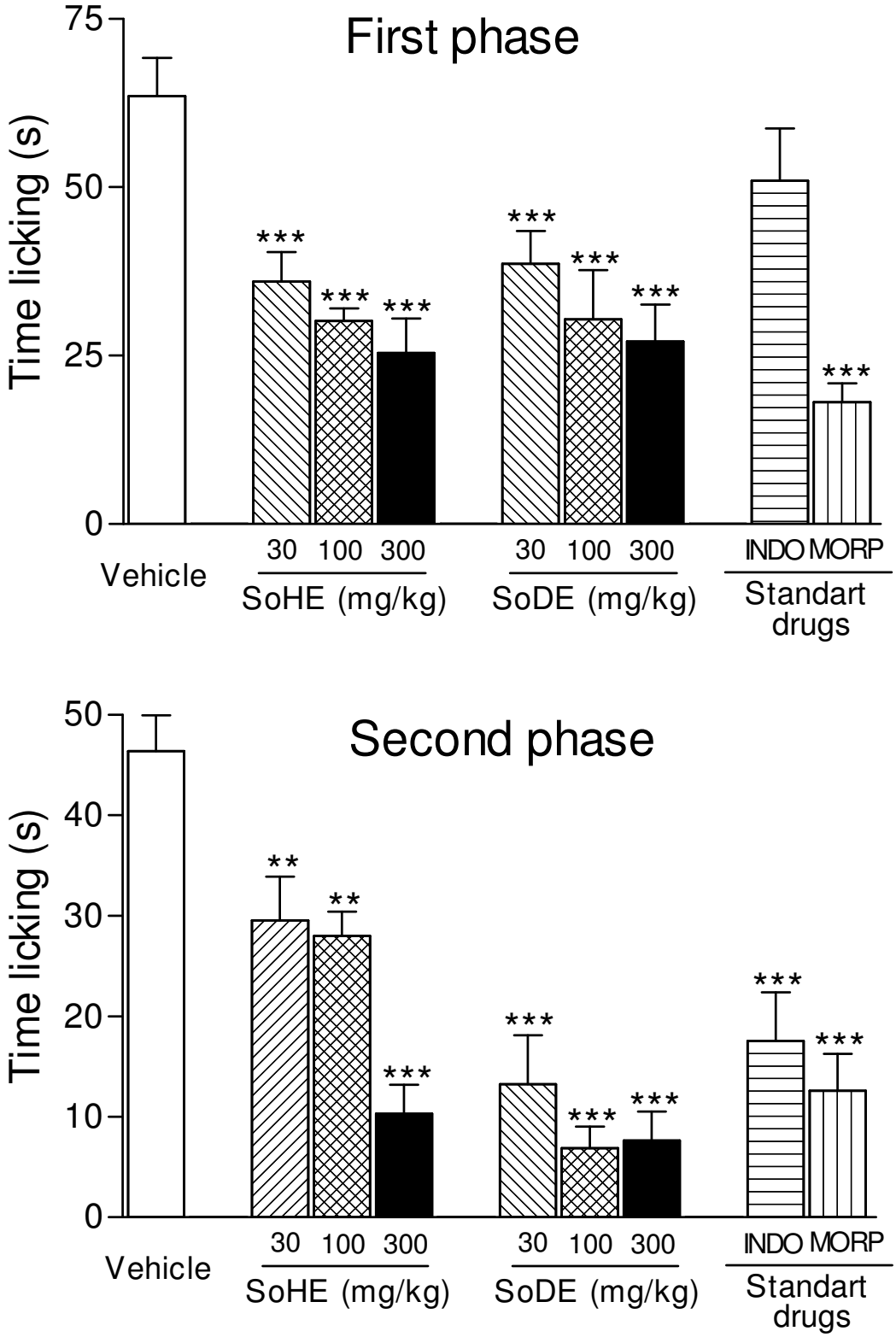


Figure 1. Vilela et al.



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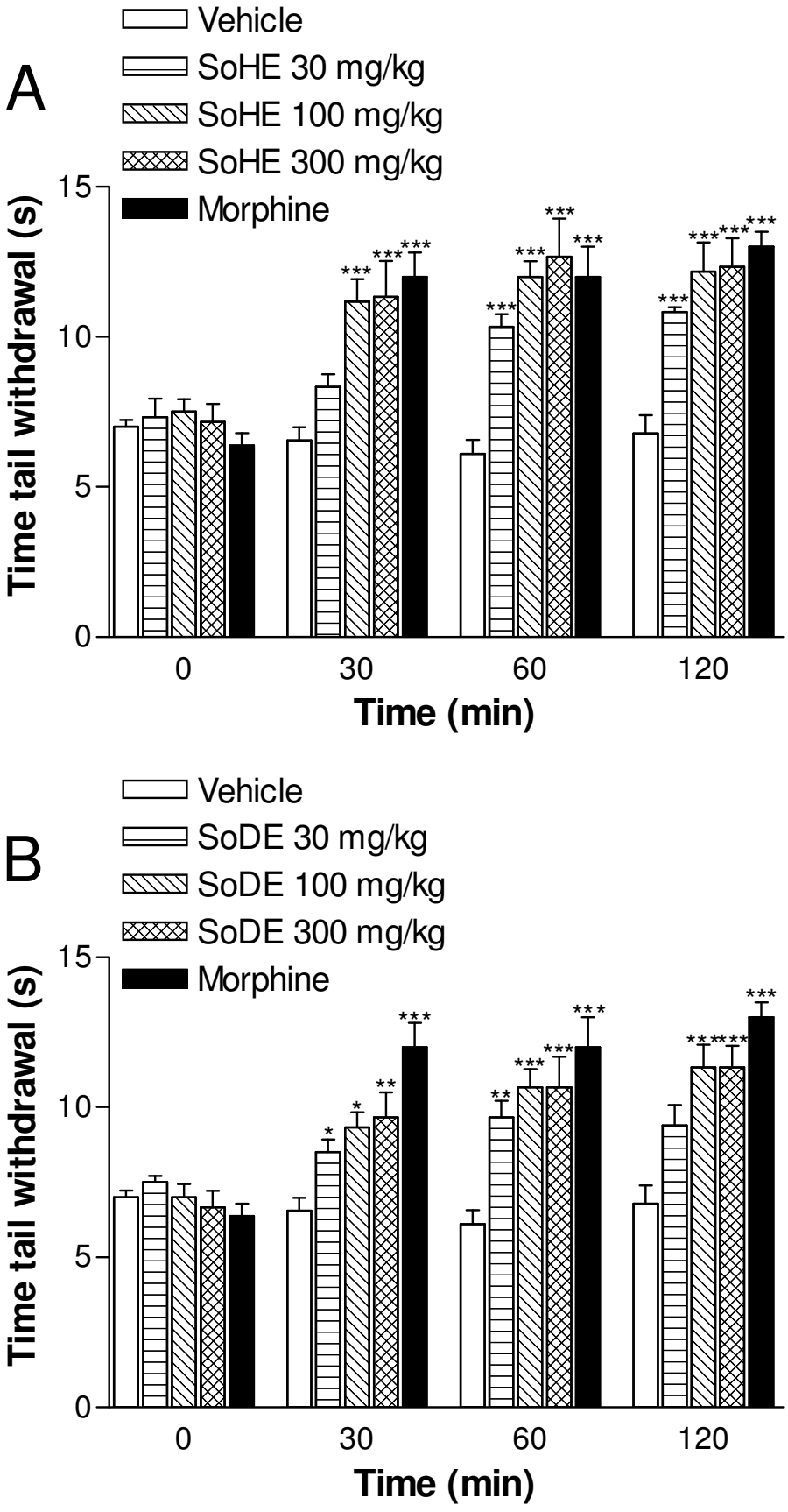

Figure 3. Vilela et al.

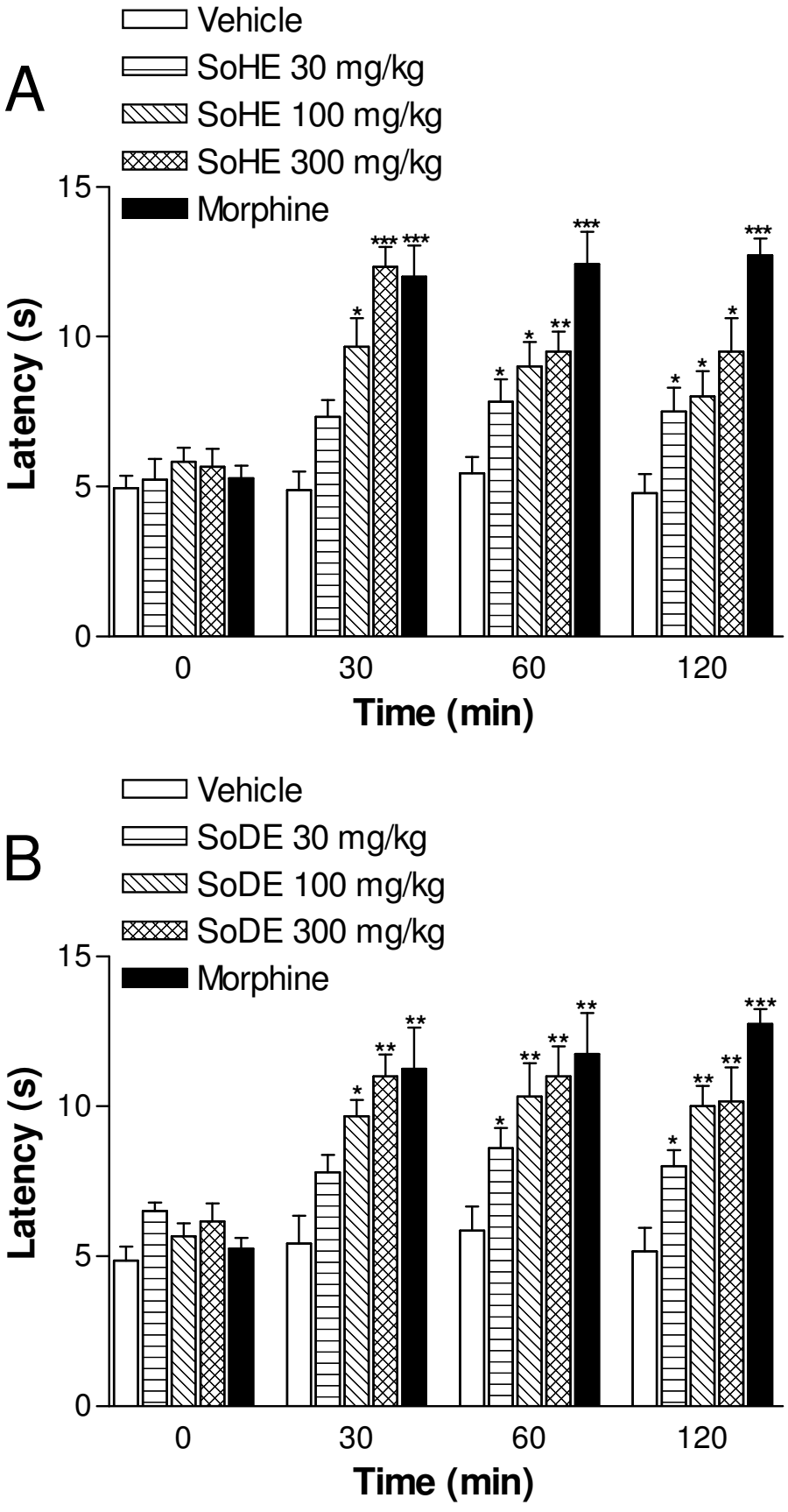

Figure 4. Vilela et al.

2.2 ARTIGO II

O artigo II descreve os materias e métodos e os resultados da avaliação da atividade ansiolítica dos extratos hidroetanólico e diclorimetânico das partes aéreas de *Sonchus oleraceus* .

O artigo foi submetido à publicação na Revista Journal of Ethnopharmacology com fator de impacto 2.049, em 19 de novembro de 2008.

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First Author: Fabiana C Vilela, MSc

Order of Authors: Fabiana C Vilela, MSc; Roseli Soncini, PhD; Alexandre Giusti-Paiva, PhD

Manuscript Region of Origin:

Alfenas–MG, November 19th, 2008

R. Verpoorte
Editor-in-Chief, Journal of Ethnopharmacology

Dear Dr. Verpoorte;

We would like to submit the manuscript entitled "**Anxiolytic-like effect of *Sonchus oleraceus* in mice**" to be considered for publication in the Journal of Ethnopharmacology. The manuscript was edited for proper English language, grammar, punctuation, spelling and overall style by native English speaking editors at American Journal Experts (key: 7D7E-C1B1-850E-80C5-B6AC).

On the basis of the traditional claim that *S. oleraceus* and the lack of scientific studies to establish its potential pharmacological properties, the objective of this study was to evaluate the antinociceptive effect of *S. oleraceus* extract in mice. All experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of Alfenas.

The manuscript, or part of it, has not been, and will not be submitted elsewhere for publication. All authors listed consented to the submission, and all data are used with the consent of the individual responsible for generating the data.

Sincerely,

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Journal of Ethnopharmacology

AUTHOR CHECKLIST

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Anxiolytic-like effect of *Sonchus oleraceus* in mice

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Abstract

Ethnopharmacological relevance: *Sonchus oleraceus* has been used as a general tonic in Brazilian folk medicine. Nevertheless, available scientific information regarding this species is scarce; there are no reports related to its possible effect on the central nervous system.

Aim of the study: This study was conducted to establish the anxiolytic effect of extracts from the aerial parts of *S. oleraceus*.

Materials and methods: This study evaluated the effect of hydroethanolic and dichloromethane extracts of *S. oleraceus* in mice submitted to the elevated plus-maze and open-field tests. Clonazepam was used as the standard drug.

Results: In the elevated plus-maze test, the *S. oleraceus* extracts increased the percentage of open arm entries and time spent in the open-arm portions of the maze. The extracts induce an anti-thigmotactic effect, evidenced by increased locomotor activity into the central part of the open field set-up. The extracts administered at 30-300 mg/kg, p.o. had a similar anxiolytic effect to clonazepam (0.5 mg/kg, p.o.).

Conclusion: These data indicate that *Sonchus oleraceus* extract exerts an anxiolytic-like effect on mice.

Keywords: Asteraceae, Open-field; Elevated plus-maze;

1. Plant.

Aerial parts of the *Sonchus oleraceus* (Asteraceae) plant were collected in March 2008 in Alfenas, Minas Gerais, Brazil. Botanical identification was conducted in the Pharmacy Department of the Federal University of Alfenas by Dr Geraldo A. da Silva. A voucher specimen was deposited in the herbarium of the Federal University of Alfenas (# 0194).

2. Uses in traditional medicine

In Brazilian traditional medicine, aerial parts of *S. oleraceus*, popularly known as "serralha," are used mostly in salad; infusion or decoction; and are administered orally to treat stomach pain, hepatitis, infections, inflammation, headaches, general pain, rheumatism, and even as a general tonic (Duarte et al., 2002; Vendruscolo and Mentz, 2006, Agra et al., 2007). In addition, the antioxidant properties of *S. oleraceus* extract have previously been reported (El and Karakaya, 2004; Schaffer et al., 2005).

3. Previously isolated components

The plant contains alkaloids, coumarins, flavonoids and saponins (Miyase and Fukushima, 1987; Ghazanfar, 1994; Guarrera et al., 2008). Taraxasterol, apigenin 7-glucuronide, and luteolin 7-glucoside have also been detected (Ghazanfar, 1994).

4. Materials and Methods

4.1. Preparation of plant extracts and reference drugs

The aerial parts of *S. oleraceus* were air-dried at 40ºC and powdered. For the hydroethanolic extract (SoHE), the dried powder was extracted by percolation with 50% ethanol at room temperature. The SoHE was concentrated until dry in a rotary evaporator and then dried with a spray dryer (BÜCHI Mini Spray Dryer B-290). For the dichloromethanic extract (SoDE),

the dried powder was extracted by percolation with dichloromethane at room temperature, and the solvent was removed under reduced pressure. The residues were used for determining bioactivity.

S. oleraceus extracts (SoHE and SoDE) were administered in 30, 100, and 300 mg/kg doses (n = 8 animals per group) after being suspended in vehicle (1% sodium carboxymethylcellulose suspension in distilled water). Clonazepan (0.5 mg/kg; Roche, Brazil) in vehicle was used as a reference drug. The tested drugs were administered orally.

4.2. Assessment of anxiolytic-like effect of *S. oleraceus* extracts

Adult male Swiss mice (22–28 g), obtained from the Central Animal Facility of the Federal University of Alfenas, were housed in a temperature-controlled room with access to water and food *ad libitum*. All experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of Alfenas.

4.2.1. Elevated plus-maze (EPM) test

The EPM consisted of two perpendicular open arms (30 cm × 5 cm) and two perpendicular closed arms (30 cm × 5cm × 25 cm). The open and closed arms were connected by a central platform (5 cm × 5 cm). The maze was 50 cm above the floor. One hour after oral treatments, the animal was placed at the center of the plus-maze with its nose in the direction of one of the closed arms. The mouse was observed for 5 min, and the following parameters were monitored: 1) number of entries in the open and closed arms, and 2) time spent by the animal in the open and closed arms (Herrera-Ruiz et al., 2008). Anxiolytic compounds reduce the animal's natural aversion to open arms and promote the exploration thereof (Pellow et al.,

1985; Lister, 1987). The apparatus was carefully cleaned with 10% ethanol solution after every test.

4.2.2. Open-field test

The open-field arena (60 cm × 60 cm) was comprised of a white floor divided into 16 squares (15 × 15 cm), enclosed by continuous 40-cm-high walls. In this test, each mouse was placed in the center of the open field, which was novel to the animal. The number of peripheral (adjacent to the walls) and central (away from the walls) squares entered by all four paws was scored for 5 min (Gomes et al., 2008). Mouse behavior was continuously videotaped by a video camera placed over the structure and then encoded using a continuous sampling method. The anti-thigmotactic effect was considered as a ratio of the number of entries into the central part as a proportion of the total entries (Prut and Belzung, 2003). The arena was carefully cleaned with 10% ethanol solution after every test.

4.3. Statistical analysis

The data obtained were analyzed using the GraphPad software program Version 4.0 and expressed as mean ± S.E.M. Statistically significant differences between groups were calculated by the application of an analysis of variance (ANOVA) followed by the Newman-Keuls test. P–values less than 0.05 ($p < 0.05$) were considered significant.

5. Results

Administration of SoHE (dose of 100 and 300 mg/kg) or SoDE (dose of 300 mg/kg) significantly increased the amount of time spent in the open arms of the EPM ($p>0.05$; Fig 1A), compared to vehicle administration. Additionally, compared to vehicle, the administration of SoHE or SoDE (both at doses of 30-300 mg/kg) promotes a significantly

greater percentage of entries into the open arms ($p<0.05$; Fig 1B), suggesting an anxiolytic effect of this preparation. The total number of arm entries was not significantly different among treatment groups (Fig 1C). Similarly, animals treated with clonazepam (0.5 mg/kg, i.p.) demonstrated a significantly increased number of entries and increased time in the open arms, as compared with controls.

The results for the open-field test are shown in Fig. 2. ANOVA demonstrated a significant effect of treatment on the number of center entries and on the ratio of central/total entries. Further analyses showed that SoHE, SoDE and clonazepam (standard drug; 0.5 mg/kg) significantly increased the number of center entries and induced an anti-thigmotactic effect, evidenced by increased locomotor activity into the central part of the open field. None of the substances tested showed a significant effect on the number of peripheral entries.

DISCUSSION

The present study investigated the putative behavioral effects in mice of the hydroethanolic and dichloromethanic extract from the aerial parts of *S. oleraceus*. The results demonstrate that SoHE and SoDE have an anxiolytic-like effect at some of the tested doses in these two murine models of anxiety.

The EPM stands as one of the most popular *in vivo* animal tests currently in use. The test was further validated as an animal model of anxiety on pharmacological, physiological and behavioral grounds (Carobrez and Bertoglio, 2005). In addition, the EPM is usually employed as a pre-clinical screening to test new anxiolytic drugs. In our study, *S. oleraceus* extracts induced an anxiolytic-like effect. Mice treated with SoHE or SoDE showed a significant increase in both the percentage of entries and the time spent in the open arms of the EPM.

The behavior of rodents in the open field depends mainly on tactile sensory factors (Prut and Belzung, 2003). Indeed, mice show thigmotaxic behavior as they lose tactile contact with the

walls (Lamprea et al., 2008). After administration of SoHE or SoDE, we observed increases in central locomotion in the central part of the device, suggesting that anti-thigmotaxic behavior can be interpreted as an anxiolytic-like effect (Prut and Belzung, 2003).

Further studies should be carried out to investigate the molecular mechanism of action of *S. oleraceus* extracts, as well as the impact on the central nervous system. However, the findings presented here do validate the traditional medicinal uses of this plant.

Acknowledgements

This work was supported by FAPEMIG, CNPq and CAPES. A. Giusti-Paiva received a research fellowship from CNPq.

References

Agra, M.F., Baracho, G.S., Nurit, K., Basílio, I.J., Coelho, V.P., 2007. Medicinal and poisonous diversity of the flora of “Cariri Paraibano”, Brazil. Journal of Ethnopharmacology 111, 383-395.

Carobrez, A.P., Bertoglio, L.J., 2005. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neuroscience Biobehavioral Reviews 29:1193-1205.

Duarte, M.G., Soares, I.A., Brandão, M., Jacome, R.L., Ferreira, M.D., Silva, C.R., Oliveira, A.B., 2002. Phytochemical screening and in vitro antibacterial activity of weed plants. Lecta 20, 177-182.

El, S.N., Karakaya, S., 2004. Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet. International Journal of Food Sciences and Nutrition 55, 67-74.

Ghazanfar, S.A., 1994. Handbook of Arabian medicinal plants. CRC Press.

Gomes, P.B., Noronha, E.C., de Melo, C.T., Bezerra, J.N., Neto, M.A., Lino, C.S., Vasconcelos, S.M., Viana, G.S., de Sousa, F.C., 2008. Central effects of isolated fractions from the root of Petiveria alliacea L. (tipi) in mice. Journal of Ethnopharmacology 120; 209-214.

Guarrera, P.M., Lucchese, F., Medori, S., 2008. Ethnophytotherapeutical research in the high Molise region (Central-Southern Italy). Journal of Ethnobiology and Ethnomedicine 4, 7.

Herrera-Ruiz, M., Román-Ramos, R., Zamilpa, A., Tortoriello, J., Jiménez-Ferrer, J.E., 2008. Flavonoids from Tilia americana with anxiolytic activity in plus-maze test. Journal of Ethnopharmacology 118, 312-317.

Lamprea, M.R., Cardenas, F.P., Setem, J., Morato, S., 2008. Thigmotactic responses in an open-field. Brazilian Journal of Medical and Biological Research 41, 135-140.

Lister, R.G., 1987. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 92, 180–185.

Miyase, T., Fukushima, S., 1987. Studies on sesquiterpene glycosides from *Sonchus oleraceus*. Chemical and Pharmaceutical Bulletin 35, 2869-2874.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open, closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Journal of Neurosciences Methods 14 1, 49–167.

Prut, L., Belzung, C., 2003. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. European Journal of Pharmacology 463, 3-33.

Schaffer, S., Schmitt-Schillig, S., Müller, W.E., Eckert, G.P., 2005. Antioxidant properties of Mediterranean food plant extracts: geographical differences. Journal of Physiology and Pharmacology 56 Suppl 1, 115-124.

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Legends.

Figure 1. Effects of hydroethanolic (SoHE) and dichloromethanic extract (SoDE) from the aerial parts of *S. oleraceus* in mice, as measured by the absolute time spent in the open arms (A), percentage of entries in the open arms (B), and number of total arm entries (C) during a 5 minute exposure to the elevated-plus-maze. Bars represents mean values (± S.E.M.) for the experimental group (n = eight animals per group). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared to vehicle group.

Figure 2. Effects of hydroethanolic (SoHE) and dichloromethanic extract (SoDE) from the aerial parts of *S. oleraceus* in mice on central entries (A), peripheral entries (B), and the ratio of central/total entries (C) during a 5 minute exposure to the open field. Bars represents mean values (± S.E.M.) for the experimental group (n = eight animals per group). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, compared to vehicle group.

Figure 1.

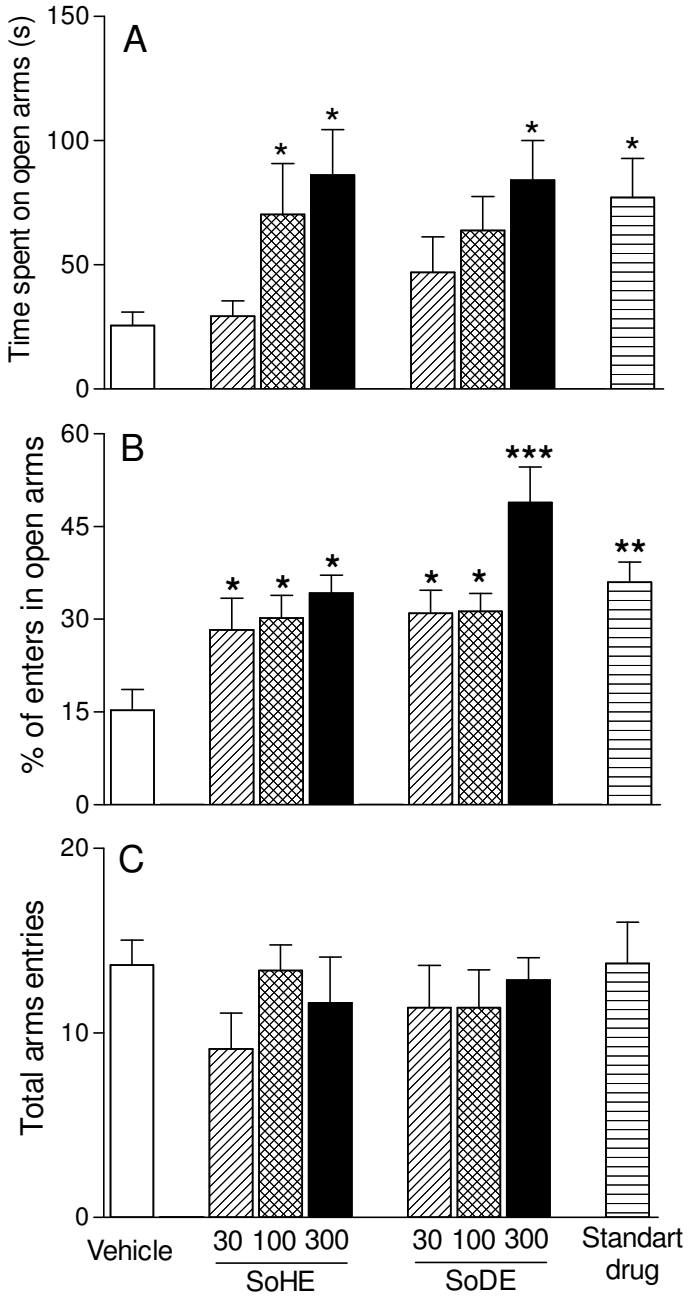

Figure 1. Vilela *et al*.

Figure 2.

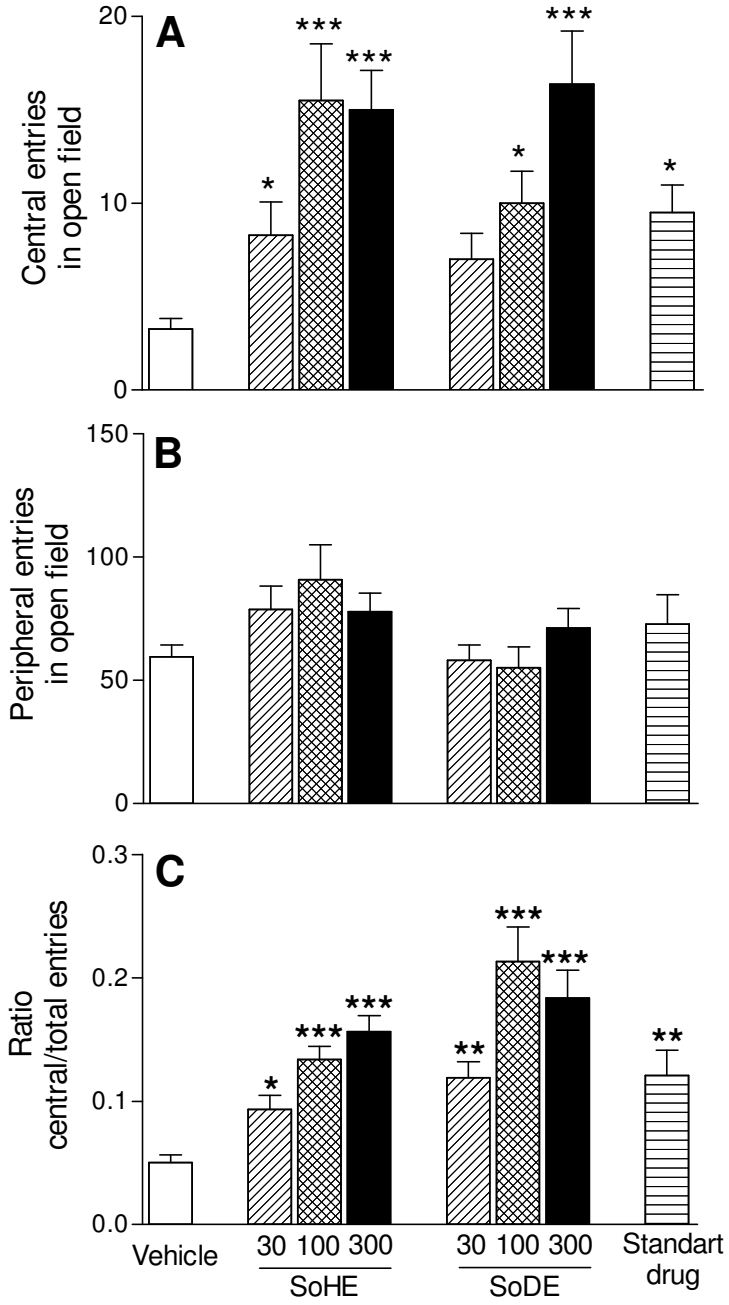

Figure 2. Vilela *et al*.

2.3 ARTIGO III

O artigo III descreve os materias e métodos e os resultados da atividade antidepressiva dos extratos hidroetanólico e diclorimetânico das partes aéreas de *Sonchus oleraceus.*

O artigo foi submetido à Revista Journal of Medicinal Food com fator de impacto 1.342, no dia 3 de dezembro de 2008.

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Antidepressant-like activity of Sonchus oleraceus in mouse models of immobility tests

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Manuscript ID:	JMF-2008-0303
Manuscript Type:	Short Communication
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Complete List of Authors:	Vilela, Fabiana; Federal University of Alfenas□MG, Biomedical Science Padilha, Marina; Federal University of Alfenas - MG, Pharmacy Alves-da-Silva, Geraldo; Federal University of Alfenas - MG, Pharmacy Soncini, Roseli; Federal University of Alfenas□MG, Biomedical Science Giusti-Paiva, Alexandre; Federal University of Alfenas - MG, Biomedical Science
Keyword:	Medicinal Food, regulatorybehavior, Bioactivity

Alfenas–MG, December 4th, 2008

Sheldon S. Hendler
Editor-in-Chief, Journal of Medicinal Food

Dear Dr. Hendler;

We would like to submit the manuscript entitled "Antidepressant-like activity of *Sonchus oleraceus* in mouse models of immobility tests" to be considered for publication in the Journal of Medicinal Food. The manuscript was edited for proper English language, grammar, punctuation, spelling and overall style by native English speaking editors at American Journal Experts (key: 1CD4-C4DC-3AF5-C003-04E6).

On the basis of the traditional claims regarding *S. oleraceus* and the lack of scientific studies to establish its potential pharmacological properties, the objective of this study was to evaluate the antidepressant effect of *S. oleraceus* extracts in mice. All experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of Alfenas.

The manuscript (or any part of it) has not been, and will not be, submitted elsewhere for publication. All authors listed consented to the submission, and all data are used with the consent of the individual responsible for generating the data.

Sincerely,

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Antidepressant-like activity of *Sonchus oleraceus* in mouse models of immobility tests

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Running title: Antidepressant effect of *Sonchus oleraceus*

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ABSTRACT

The aim of the present work is to evaluate the putative antidepressant-like effects of a hydroethanolic and dichloromethanic extract from the aerial parts of *Sonchus oleraceus* (Family: Asteraceae) on the performance of male mice in the forced swimming test (FST) and tail suspension test (TST) models predictive of depression.

The hydroethanolic and dichloromethanic extracts, both in doses of 30, 100 and 300 mg/kg, were orally administered (p.o.) one hour before carrying out FST or TST. The immobility time in both FST and TST was significantly reduced by acute oral treatment with the extracts (dose range 100–300 mg/kg), without accompanying changes in ambulation, as assessed in an open-field test. This excluded the possibility that the effect of the extracts is due to an activation of locomotion. The efficacy of the extracts was found to be comparable to that of amitriptyline (10 mg/kg, p.o.).

The present study provides evidence for an antidepressant-like effect of the active principle(s) present in the extracts of *S. oleraceus* in mice. Therefore, a standardized *S. oleraceus* extract or its purified constituents could be of potential interest for the treatment of depressive disorders.

KEY WORDS: depression; forced swimming test; tail suspension test; Asteraceae.

INTRODUCTION

Depressive disorders represent a major public health problem owing to their high prevalence and psychosocial impact.[1] People from different regions of the world have used herbal medicines to alleviate affective disorders.[1,2] Herbal therapies may be effective alternatives in the treatment of depression, and the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses, including depression, has progressed significantly in the past decade.[2,3]

Sonchus oleraceus L. (Asteraceae) is edible to humans as a leaf vegetable and is frequently consumed in Mediterranean countries,[4-8] Australia[9] and New Zealand, particularly by the native Maori.[10] South of Minas Gerais in Brazil (Southeast of Brazil), *S. oleraceus*, known as “serralha”, is very important in the diets of rural people, especially seasonally, and provides cash income to the harvester and relatively inexpensive vegetables to the community.

In traditional Brazilian medicine, the aerial parts of *S. oleraceus* are used mostly in infusion or decoction, and are administered orally for treating pain, headaches, and as a general tonic.[11-13]

Based on the traditional claims surrounding *S. oleraceus* and the lack of scientific studies of its potential pharmacological properties, the objective of this study was to examine the antidepressant-like effect of the extract of *S. oleraceus* in the mouse tail suspension test and forced swimming test, predictive models of antidepressant activity.[14, 15]

MATERIALS AND METHODS

Animals

Adult male Swiss mice (22–30 g), obtained from the Central Animal Facility of the Federal University of Alfenas, were housed in a temperature-controlled room with access to water and food *ad libitum*. All experiments were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of Alfenas.

Preparation of plant extracts and reference drugs

The aerial parts of *S. oleraceus* were air-dried at 40ºC and powdered. For the hydroethanolic extract (SoHE), the dried powder was extracted by percolation with 50% ethanol at room temperature. The SoHE was concentrated in a rotary evaporator and then dried with a spray dryer (BÜCHI Mini Spray Dryer B-290). For the dichloromethanic extract (SoDE), the dried powder was extracted by percolation with dichloromethane at room temperature, and the solvent was removed under reduced pressure. The residues were used for determining bioactivity.

Animals were treated with the SoHE or SoDE (30, 100 and 300 mg/kg; n = 8 animals per group) or with vehicle (1% sodium carboxymethylcellulose suspension in distilled water) given by the p.o. 1 h before the experiments. Amitriptyline (10 mg/kg) in vehicle was used as a reference drug. The tested drugs were administered orally.

Forced swimming test (FST)

Mice were individually placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with water to a depth of 12 cm (28 ± 1ºC). All animals were forced to swim for 6 min, and the duration of immobility was observed and measured during the final 4 min interval of

the test.[16] All test swim sessions were recorded by a video camera positioned directly above the cylinder. The immobility period was regarded as the time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above the water.

Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to a previously method described.[14] Briefly, mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period.[17]

Locomotor activity test

To assess the possible effects of the SoHE and SoDE on locomotor activity, mice were evaluated in the open-field paradigm as previously described.[18] Mice were individually placed in a box (60×60×50 cm) with the floor divided into 16 squares. The number of squares crossed with the four paws was registered during a period of 5 min.

Statistical analysis

The data obtained were analyzed using the GraphPad software program Version 4.0 and expressed as mean ± S.E.M. Statistically significant differences between groups were calculated by the application of an analysis of variance (ANOVA) followed by the Newman-Keuls test. P–values less than 0.05 ($p < 0.05$) were considered significant.

RESULTS AND DISCUSSION

In the present study, hydroethanolic and dichloromethanic extracts of *S. oleraceus* produced a significant antidepressant-like effect in mice as assessed by both the FST and the TST. Furthermore, the effect of the extracts of *S. oleraceus* in the FST and TST was similar to the effect produced by the oral administration of amitriptyline, used as a positive control. Both the FST and TST are used routinely to characterize antidepressant activity of a wide variety of test compounds.[14-21]

In the FST, mice are forced to swim in a restricted space from which they cannot escape, and are induced to assume a characteristic behavior of immobility. This behavior reflects a state of despair, which can be reduced by several agents that are therapeutically effective in human depression. The administration of SoHE or SoDE significantly reduces the time of immobility in FST (Fig. 1). The results from the FST indicate that at doses 100 and 300 mg/kg, SoHE and SoDE significantly reduced the duration of immobility compared with vehicle control. In this regard, the effect of the two doses was indistinguishable as they produced a comparable reduction of immobility in swimming.

The TST also induces a state of immobility in animals, in a similar fashion to that of the FST. This immobility is often referred to as behavioral despair in animals.[14,22] The results of this study (reduction of immobility time) provide evidence that orally administered extracts of *S. oleraceus* produce an antidepressant-like effect in the TST (Fig. 1).

The antidepressant-like effect of *S. oleraceus* extracts seems not to be associated with any motor effects, since they had no effect on locomotor activity of mice as compared with the control (Fig. 2). This indicates that increased motor activity was not involved in the antidepressant-like action of the extracts in either the FST or the TST, and confirms that the antidepressant-like effect is specific.

In summary, our data indicate an antidepressant-like effect of the hydroethanolic and dichloromethanic extracts of *S. oleraceus*, since the reduction of immobility time elicited by its administration cannot be attributable to any psychostimulant effect. Phytochemical studies have identified active components of *S. oleraceus* such as taraxasterol, apigenin 7-glucuronide, and luteolin 7-glucoside.[23] Alkaloids, coumarins, flavonoids and saponins have also been detected.[7,23,24] However, a preliminary characterization of the extracts used in the present study showed the presence of alkaloids, phenolic compounds, triterpenoids, steroids and tannins in both the SoHE and the SoDE. Further chemical analysis of the extract will be conducted to isolate and characterize the active principles responsible for the observed effects.

Since that use of *S. oleraceus* is prevalent, especially in rural areas,[11-13] In order to support appropriate and safe use of these medicines, it is essential to collect pharmacological evidence for the action and the underlying mechanisms of these extracts. Whereas the precise mechanisms by which *S. oleraceus* extracts produced antidepressant-like activity are not completely understood, they may have potential therapeutic value for the management of depressive disorders.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

REFERENCES

1. Carlini EA: Plants and the central nervous system. Pharmacol Biochem Behav 2003;75:501-512.

2. Zhang ZJ: Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci 2004;75:1659-1699.

3. Sarris J: Herbal medicines in the treatment of psychiatric disorders: a systematic review. Phytother Res 2007;21:703-716.

4. Guil-Guerrero JL, Gimenez-Gimenez A, Rodriguez-Garcia I, Torija-Isasa ME, Nutritional composition of *Sonchus* species (*S. asper* L, *S. oleraceus* L and *S. tenerrimus* L). J Sci Food Agric 1998;76:628-632.

5. Guarrera PM: Food medicine and minor nourishment in the folk traditions of Central Italy (Marche, Abruzzo and Latium). Fitoterapia 2003;74:515-544.

6. Guarrera PM, Salerno G, Caneva G: Food, flavouring and feed plant traditions in the Tyrrhenian sector of Basilicata, Italy. J Ethnobiol Ethnomed 2006;2:37.

7. Guarrera PM, Lucchese F, Medori S: Ethnophytotherapeutical research in the high Molise region (Central-Southern Italy). J Ethnobiol Ethnomed 2008:4;7.

8. Lentini F, Venza F: Wild food plants of popular use in Sicily. J Ethnobiol Ethnomed 2007;3:15.

9. Liu L, Howe P, Zhou YF, Hocart C, Zhang R: Fatty acid profiles of leaves of nine edible wild plants: an Australian study. J Food Lipids 2007;9: 65-71.

10. Cambie RC, Ferguson LR: Potential functional foods in the traditional Maori diet. Mutat Res 2003;523-524:109-117.

11. Duarte MG, Soares IA, Brandão M, Jacome RL, Ferreira MD, Silva CR, Oliveira AB: Phytochemical screening and in vitro antibacterial activity of weed plants. Lecta 2002;20:177-182.

12. Vendruscolo GS, Mentz AZ: Ethnobotanical survey of the medicinal plants used by the community of Ponta Grossa neighborhood, Porto Alegre, Rio Grande do Sul, Brazil. Iherigia Série Botânica 2006;61:83-103.

13. Agra MF, Baracho GS, Nurit K, Basílio IJ, Coelho VP: Medicinal and poisonous diversity of the flora of "Cariri Paraibano", Brazil. J Ethnopharmacol 2007;111:383-395.

14. Steru L, Chermat R, Thierry B, Simon P: The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 1985;85:367-370.

15. Petit-Demouliere B, Chenu F, Bourin M: Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology 2005;177:245-255.

16. Porsolt RD, Le Pichon M, Jalfre M: Depression: a new animal model sensitive to antidepressant treatments. Nature 1977;266:730–732.

17. Mantovani M, Pértile R, Calixto JB, Santos AR, Rodrigues AL: Melatonin exerts an antidepressant-like effect in the tail suspension test in mice: evidence for involvement of N-methyl-D-aspartate receptors and the L-arginine-nitric oxide pathway. Neurosci Lett 2003; 343:1-4.

18. Rodrigues AL, da Silva GL, Mateussi AS, Fernandes ES, Miguel OG, Yunes RA, Calixto JB, Santos AR: Involvement of monoaminergic system in the antidepressant-like effect of the hydroalcoholic extract of *Siphocampylus verticillatus*. Life Sci 2002;70:1347-1358.

19. Porsolt RD, Anton G, Deniel M, Jalfre M: Behavioral despair in rats: a new animal model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47:379–391.

20. Dhingra D, Sharma A: Antidepressant-like activity of n-hexane extract of nutmeg (Myristica fragrans) seeds in mice. J Med Food 2006;9:84-89.

21. Anjaneyulu M, Chopra K, Kaur I: Antidepressant activity of quercetin, a bioflavonoid, in streptozotocin-induced diabetic mice. J Med Food 2003;6:391-395.

22. Thierry B, Steru L, Simon P, Porsolt RD: The tail suspension test: ethical considerations. Psychopharmacology 1986;90:284–285.

23. Rizk AM: Constituents of plants growing in Qatar I. A chemical survey of sixty plants. Fitoterapia 1982;53:35-44.

24. Miyase T, Fukushima S: Studies on sesquiterpene glycosides from *Sonchus oleraceus*. Chem Pharm Bull 1987;35:2869-2874.

LEGENDS

Figure 1. Effect of treatment with hydroethanolic (SoHE) or dichloromethanic (SoDE) extract of *S. oleraceus* or standard drug (amitriptylin), given orally, on the immobility of mice in the forced swimming test (top) and tail suspension test (bottom). Each column represents the mean ± S.E. of 8 animals per group. *P<0.05; **P<0.01; ***P<0.001 compared with the vehicle-group.

Figure 2. Effect of treatment with hydroethanolic (SoHE) or dichloromethanic (SoDE) extract of *S. oleraceus* or standard drug (amitriptylin), given orally, on locomotor activity evaluated in the open field test. Each column represents the mean ± S.E. of the number of squares crossed in 5 min of locomotor activity test (n = 8 animals per group).

Fig. 1

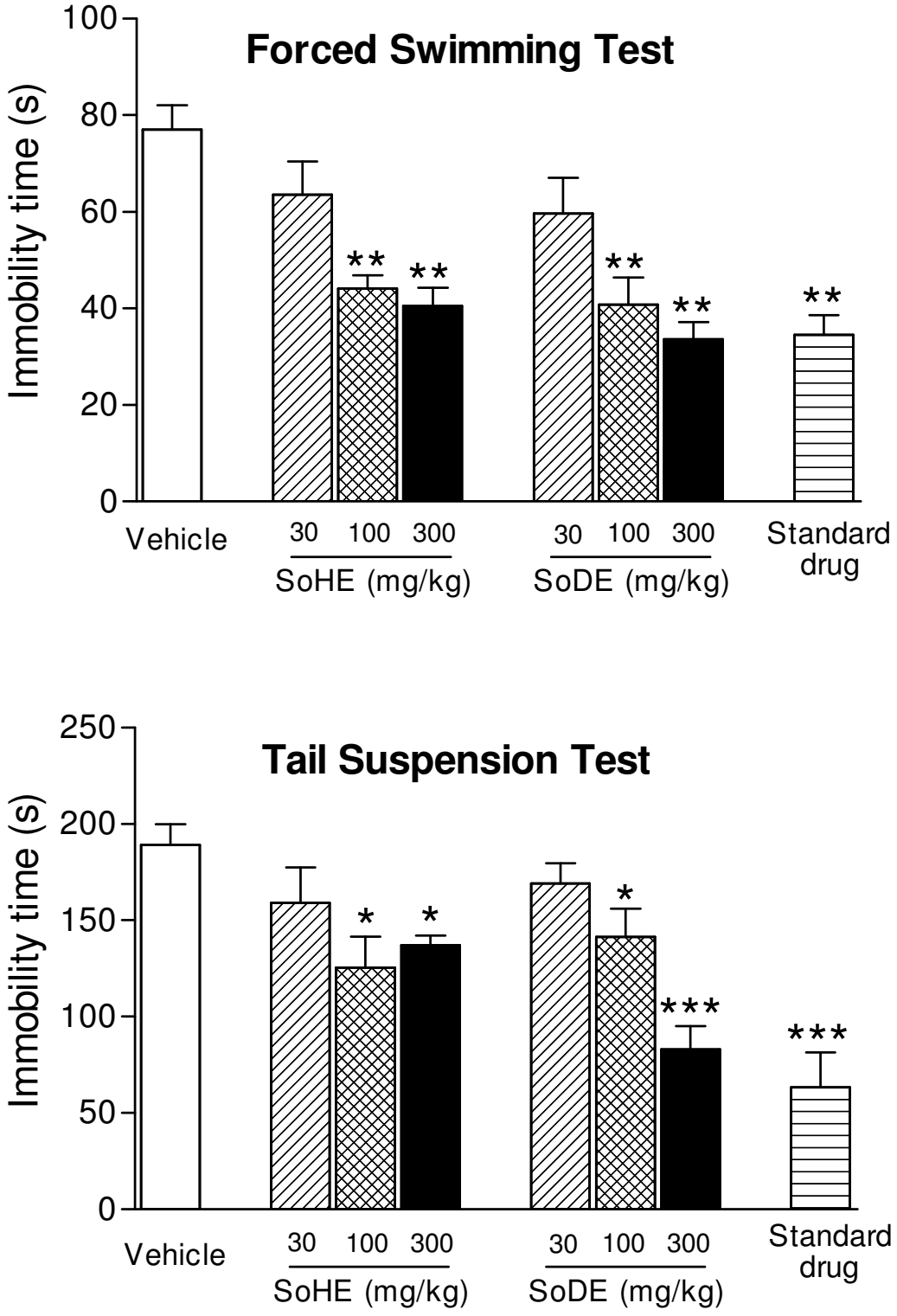
Forced Swimming Test
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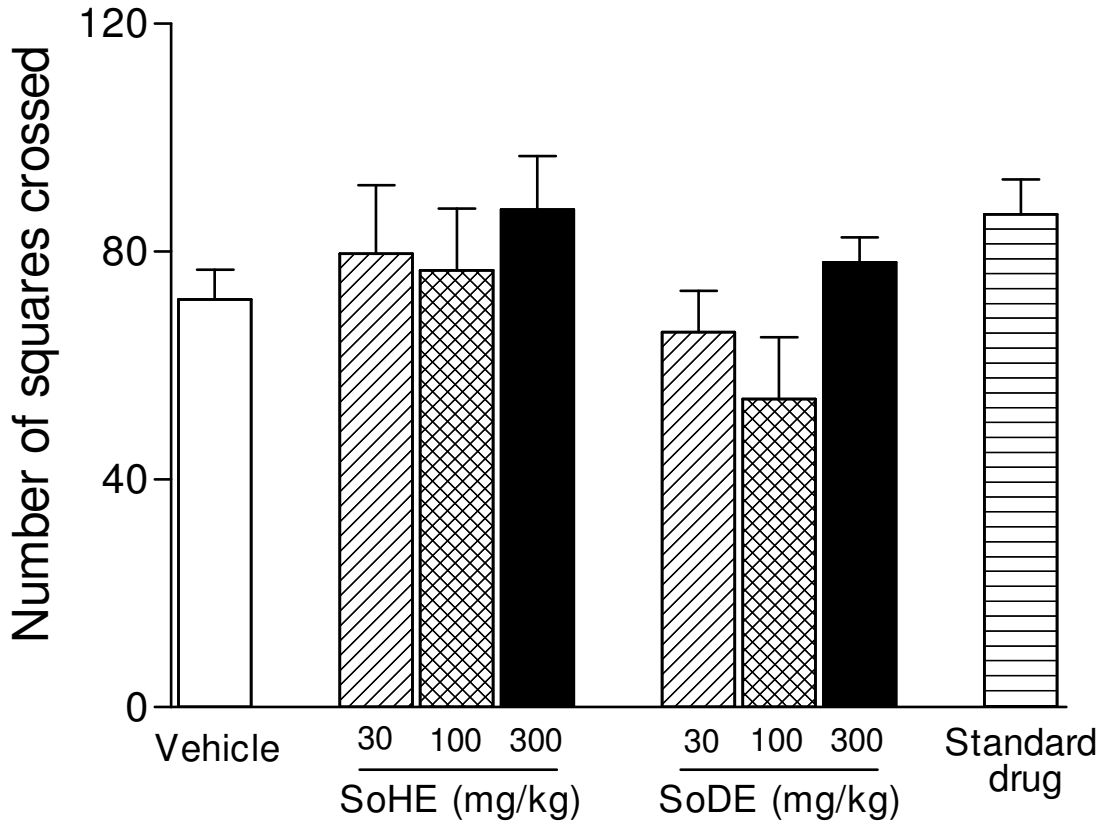
Figure 2.



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3 CONCLUSÃO

O trabalho mostrou o efeito dos extratos hidroetanólico e diclorometânico das partes aéreas de *S. oleraceus* em diferentes respostas antinociceptivas geradas por estímulo nocivo químico produzido por injeção de formalina ou ácido acético, ou por estímulo nocivo térmico nos testes de placa quente e imersão da cauda. Este efeito pode estar associado à presença de metabólitos secundário presentes na espécie em estudo, como flavonóides e triterpenos.

Os extratos também induziram ao efeito ansiolítico, uma vez que os animais tratados com os extratos mostraram um aumento significante em ambas às porcentagens de entrada e tempo nos braços abertos do labirinto em cruz elevado. Observou-se também um aumento de locomoção dos animais no centro do campo aberto sugeriu um comportamento anti-tigmotáxico que pode ser interpretado como um efeito ansiolítico, semelhante ao clonazepam.

Os dados dos testes antidepressivos indicaram um efeito antidepressivo dos extratos, uma vez que reduziram o tempo de imobilidade no teste de suspensção pela cauda e nado forçado. Esse efeito parece estar associado a nenhum efeito motor, uma vez que os animais tratados não tiveram um aumento de atividade locomotora quando comparada ao controle no teste do campo aberto, descartando assim um possível efeito psicoestimulante. Estudos demonstraram que flavonóides podem ser substâncias com atividade antidepressiva e *S. oleraceus* possui esse metabólito secundário.

Como *S. oleraceus* é conhecida e usada popularmente, é essencial esclarecer os mecanismos de ação dos extratos bem como isolar substâncias que possam ser responsáveis pelos efeitos apresentados nesse trabalho.