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ESTUDO DA AÇÃO ANTIPSICÓTICA E EFEITOS COLATERAIS DO HALOPERIDOL NANOENCAPSULADO EM RATOS

TESE DE DOUTORADO

Dalila Moter Benvegnú

Santa Maria, RS, Brasil

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ESTUDO DA AÇÃO ANTIPSICÓTICA E EFEITOS COLATERAIS DO HALOPERIDOL NANOENCAPSULADO EM RATOS

Dalila Moter Benvegnú

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Farmacologia, Área de Concentração em Neuropsicofarmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
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Orientadora: Profª. Dra. Marilise Escobar Bürger

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como requisito parcial para obtenção do grau de
Doutor em Farmacologia

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"A vida é uma peça de teatro que não permite ensaios. Por isso, cante, chore, dance, ria e viva intensamente, antes que a cortina se feche e a peça termine sem aplausos."

Charles Chaplin

Dedico esta tese aos meus
queridos pais, Valter e Leda.

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Farmacologia
Universidade Federal de Santa Maria, RS, Brasil

ESTUDO DA AÇÃO ANTIPSICÓTICA E EFEITOS COLATERAIS DO HALOPERIDOL NANOENCAPSULADO EM RATOS

Autora: Dalila Moter Benvegnú
Orientadora: Profª. Drª. Marilise Escobar Bürger
Data e Local da Defesa: Santa Maria, 29 de junho de 2012

O haloperidol é um neuroléptico típico comumente utilizado no tratamento de desordens psicóticas e continua sendo o antipsicótico mais prescrito no mundo, por sua elevada potência e baixo custo. Apesar da boa eficácia terapêutica, o uso do fármaco é relacionado ao desenvolvimento de sérios efeitos adversos de natureza endócrina, cognitiva e motora. De particular importância, os distúrbios motores podem se manifestar de forma irreversível e incapacitante e sua fisiopatologia tem sido associada à geração de radicais livres, estresse oxidativo e morte neuronal dopaminérgica em regiões extrapiramidais. Atualmente, as nanopartículas poliméricas constituem uma importante ferramenta na farmacologia, pois possibilitam uma vetorização e liberação controlada de fármacos, o que pode aumentar a eficácia terapêutica e reduzir os efeitos adversos. Neste contexto, o presente estudo teve como objetivo geral fazer uma avaliação comparativa entre formulações de haloperidol nanoencapsulado e o fármaco livre, considerando sua atividade terapêutica e efeitos colaterais em ratos. Inicialmente, foram desenvolvidas suspensões contendo nanocápsulas de haloperidol, cuja caracterização físico-química mostrou que o fármaco foi satisfatoriamente nanoencapsulado. Após um primeiro estudo utilizando um modelo animal de pseudo-psicose, a formulação de haloperidol nanoencapsulado mostrou superioridade terapêutica qualitativa e quantitativa, observadas através da maior eficácia e maior tempo de ação antipsicótica. Em relação aos efeitos colaterais extrapiramidais, a formulação nanoencapsulada foi capaz de prevenir e/ou minimizar os distúrbios motores agudos e subcrônicos característicos, os quais são frequentemente observados com o fármaco livre. Com o objetivo de otimizar os resultados até então observados, foi desenvolvida uma nova formulação contendo nanocápsulas de haloperidol a partir da substituição do núcleo oleoso composto por triglicerídeos cápricos e caprílicos por óleo de peixe, o qual é rico em ácidos graxos poliinsaturados e tem sido descrito por suas propriedades neuroprotetoras e antiapoptóticas. Esta formulação mostrou adequabilidade físico-química e redução de efeitos adversos motores em relação ao fármaco livre; demonstrando também capacidade de atuar na redução do estresse oxidativo, observado através dos menores níveis de peroxidação lipídica, bem como aumento das defesas antioxidantes em regiões extrapiramidais. Uma terceira etapa experimental foi desenvolvida a partir de uma nova formulação de haloperidol contendo óleo de semente de uva em substituição aos ácidos graxos de cadeia média acima referidos, a qual não atingiu completamente as propriedades necessárias para uma nanoformulação, mas mesmo assim foi incluída no estudo comparativo entre as diferentes formulações de nanocápsulas. Neste estudo, a nanoformulação de haloperidol com óleo de peixe mostrou maior capacidade de prevenir os efeitos colaterais motores comuns com o fármaco livre, atuando na manutenção da viabilidade celular e no controle da geração de radicais livres. Tomados em conjunto, os resultados do presente estudo apontam de forma favorável quanto ao desenvolvimento de formulações de nanocápsulas contendo haloperidol, o que caracteriza uma perspectiva positiva para a minimização de efeitos adversos motores decorrentes do uso do fármaco livre. Neste sentido, estas formulações poderiam colaborar na redução das limitações físicas incapacitantes e causadoras de constrangimento frequentemente descritas, contribuindo para uma melhor qualidade de vida dos pacientes psiquiátricos.

Palavras-chave: Haloperidol. Nanocápsulas Poliméricas. Estresse Oxidativo. Efeitos Extrapiramidais.

ABSTRACT

Doctoral Thesis
Post-Graduate Program in Pharmacology
Federal University of Santa Maria, RS, Brasil

ANTIPSYCHOTIC ACTION AND ADVERSE SIDE EFFECTS STUDY OF HALOPERIDOL-LOADED NANOCAPSULE IN RATS

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Advisor: Marilise Escobar Bürger
Date and place of defense: June 29th, 2012, Santa Maria

Haloperidol is a typical neuroleptic widely used in the treatment of several psychotic disorders and it remains the most prescribed antipsychotic worldwide, due to its high potency and low cost. Despite its good therapeutic efficacy, the use of this drug is related to development of serious side effects of endocrine and cognitive nature, besides movement disturbances. Of particular importance, the motor disorders may be manifested in an irreversible and disabling form, whose pathophysiology has been linked to free radical generation, oxidative stress and dopaminergic neuronal death in extrapyramidal regions. Currently, polymeric nanoparticles are important terapeutic tools in the pharmaceutical area, because they permit a vectorization and a control drug release, it increasing the therapeutic efficacy and reducing adverse side effects. In this context, the present study aimed to perform a comparative evaluation among formulations of haloperidol-loaded nanocapsules and the free drug considering to its therapeutic action and side effects in rats. Firstly, it was prepared suspensions containing nanocapsules of haloperidol whose physicochemical characterization showed that haloperidol can be satisfactory encapsulated. After a first study using a pseudo-psychosis animal model, the haloperidol nanoencapsulated formulation demonstrated qualitative and quantitative therapeutic superiority, which were observed by the higher eficacy and action duration. In relation to extrapyramidal side effects, encapsulated formulation was able to prevent and/or minimize the characteristic acute and chronic movement disturbances, frequently observed with the free drug. In order to improve the results already obtained, a new formulation containing haloperidol nanocapsules was developed from the replacement of the oily core composed of caprylic and capric triglycerides in fish oil rich in polyunsaturated fatty acids, that has been described by its neuroprotective and antiapoptotic properties. This formulation showed physicalchemical suitability and reduced side effects in relation to the free drug, showing also ability to act in the prevention of oxidative stress which was observed by lower levels of lipid peroxidation, as well as an increase of antioxidant defenses in extrapyramidal regions. A third experimental phase was developed from the preparation of a new formulation of haloperidol containing grape seed oil in place of medium chain fatty acids mentioned above, which did not reach the necessary properties for a nanoformulation, but it was included for the comparative study of different nanocapsules formulations. The nanoformulation of haloperidol containing fish oil showed higher ability to prevent the motor side effects commonly seen with the free drug, which were observed by the maintenance of cell viability and control of free radical generation. Taken together, the results of this study point favorably to the development of nanocapsules formulations containing haloperidol, characterizing a positive perpective for minimizing of motor side effects consequent to the free drug. In this sense, these formulations can reduce the disabling physical limitations that cause embarrassment, contributing to a better quality of life of psychiatric patients.

Key-words: Haloperidol. Polymeric Nanocapsules. Oxidative Stress. Extrapyramidal Effects.

LISTA DE ILUSTRAÇÕES

REVISÃO BIBLIOGRÁFICA

ARTIGO 1

MANUSCRITO 1

MANUSCRITO 2

LISTA DE TABELAS

LISTA DE ABREVIATURAS

BHE – barreira hematoencefálica
CAT – catalase
DA – dopamina
DO – discinesia orofacial
DT – discinesia tardia
EO – estresse oxidativo
EROs – espécies reativas de oxigênio
GSH – glutationa reduzida
LPO – peroxidação lipídica ou lipoperoxidação
PCL – poli(ε-caprolactona)
RL – radicais livres
SNC – sistema nervoso central
TBARS – substâncias reativas ao ácido tiobarbitúrico
TCM – triglicerídeos de cadeia média

SUMÁRIO

APRESENTAÇÃO

Esta tese de doutorado está estruturada em seções dispostas da seguinte forma: Introdução, Objetivos, Artigos Científicos, Discussão, Conclusões, Perspectivas e Referências Bibliográficas.

Os itens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas encontram-se inseridos nos próprios artigos na seção **MANUSCRITOS CIENTÍFICOS** e representam a íntegra deste estudo.

Ao fim desta tese, encontram-se os ítens **DISCUSSÃO** e **CONCLUSÕES**, nos quais há interpretações e comentários gerais sobre os artigos científicos contidos neste estudo.

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

1 INTRODUÇÃO

1.1 Psicoses e esquizofrenia

O termo psicose descreve os distúrbios psiquiátricos graves, geralmente de origem desconhecida ou idiopática, portanto, "funcionais", nos quais são encontrados, além dos distúrbios do comportamento, a incapacidade de pensar coerentemente e de compreender a realidade. A orientação e a memória estão conservadas, apesar do comprometimento do pensamento, das emoções e do comportamento (SILVA, 2002). As psicoses apresentam uma prevalência ao longo da vida de 2-3% e contam com elevada percentagem de morbidades severas associadas a doenças psiquiátricas. O que define as características das psicoses são os sintomas psicóticos, como uma ilusão (uma crença, apesar de ser provado o contrário), uma alucinação (experiência sensorial sem um estímulo externo) ou pensamento desordenado (CROW; HARRINGTON, 1994; FLETCHER; FRITH, 2009).

O termo "esquizofrenia" (esquizo = divisão; phrenia = mente), cunhado pela primeira vez, em 1908, por Eugen Bleuler (1857-1930), faz alusão, como a própria etimologia da palavra indica, a uma quebra entre as funções do pensamento, da afetividade e do comportamento. Mais de oitenta anos após a criação desta denominação, a doença ainda não representa uma entidade única definida, em função da heterogeneidade de suas formas de apresentação (BRANDÃO; GRAEFF, 1996). Bleuler classificou ainda os sintomas da esquizofrenia, conforme suas características, em sintomas positivos, os quais incluem delírios e alucinações e negativos, como perda motivacional e oscilação emocional (LEWIS; LIEBERMAN, 2000; SAWA; SNYDER, 2002).

A esquizofrenia consiste na desordem mental mais crônica, incapacitante e custosa (MAHADIK et al., 2001), afetando cerca de 1% da população mundial (OMS, 2000; APA, 2004), ou seja, apresentando uma incidência semelhante a do *diabetes mellitus* (HARVEY; CHAMPE, 2002), independente da cultura, país ou grupo racial (BROMET; FENNING, 1999). Estima-se que, somente no Brasil, cerca de 1.170.000 pessoas sofram de esquizofrenia e cerca de 80.000 novos casos acabem surgindo anualmente (THEME-FILHA et al., 2005). Esta doença apresenta um forte componente genético e as manifestações surgem, geralmente, entre o final da segunda década de vida e o início da terceira, caracterizando-se por delírios, alucinações, afeto embotado, desorganização do pensamento e distúrbios da fala (SILVA, 2006).

A etiologia da esquizofrenia ainda continua não completamente esclarecida. Embora existam várias hipóteses bioquímicas desenvolvidas para explicar a gênese da doença, como a hipótese glutamatérgica e a serotonérgica, a hipótese da hiperfunção dopaminérgica central atualmente é a mais bem investigada e mais aceita. No entanto, sabe-se que, além do sistema dopaminérgico, outros sistemas de neurotransmissores centrais desempenham algum papel, sendo provável seu envolvimento simultâneo (LIEBERMAN et al., 1998).

A hipótese dopaminérgica da esquizofrenia postula que os sintomas positivos da doença sejam secundários a uma hiperatividade dopaminérgica subcortical ou, mais precisamente, mediados pelos receptores dopaminérgicos do tipo D_2 da via dopaminérgica mesolímbica (LARUELLE et al., 1999). A revisão da hipótese dopaminérgica atribui também um déficit de atividade da via dopaminérgica mesocortical (DAVIS et al., 1991; KNABLE; WEINBERGER, 1997; BOWIE; HARVEY, 2006) ou, em específico, uma hipoatividade do receptor dopaminérgico do tipo D_1 no córtex-pré-frontal (ABI-DARGHAM, 2004). Essa hipofunção dopaminérgica estaria relacionada com os sintomas negativos e cognitivos observados na doença (DAVIS et al., 1991; KNABLE; WEINBERGER, 1997; GOLDMAN-RAKIC et al., 2004). Em suma, acredita-se atualmente que um déficit na atividade dopaminérgica cortical e um aumento na atividade dopaminérgica subcortical estariam envolvidos na doença (ABI-DARGHAM, 2004; LARUELLE, 2005; HOWES; KAPUR, 2009).

Dois conjuntos de observações confirmam a hipótese dopaminérgica: primeiramente, provando que a eficácia dos neurolépticos está correlacionada com a sua ligação aos receptores D_2, antagonizando os efeitos da dopamina (DA) (SEEMAN, 1975; 1976; CREESE, 1976) e que agonistas potentes dos receptores D_2, como a apomorfina, são capazes de induzir surtos psicóticos em animais (VÕIKAR et al., 1999; MOTAMAN et al., 2005; MUTHU; SINGH, 2008). Em segundo lugar, a observação de que estimulantes como a anfetamina e a cocaína, quando administrados cronicamente, induzem ao aumento da atividade catecolaminérgica central, podendo desencadear episódios psicóticos (ROBINSON; BECKER 1986; LYON, 1991; BYRNES; HAMMER, 2000; CURRAN et al., 2004).

1.2 Fármacos antipsicóticos

Os antipsicóticos estão entre os psicotrópicos mais prescritos, constituindo uma farmacoterapia amplamente empregada no controle dos sintomas psicóticos e em distúrbios do comportamento associados à demência e drogas de adição (RAJA, 1995; ARRUDA et al.,

2012). Historicamente, os antipsicóticos passaram a ser utilizados na psiquiatria a partir da descoberta casual de Delay e Deniker, no início da década de 50, quando a prometazina, um potente anti-histamínico mostrou que sua propriedade sedativa era acompanhada de indiferença, diminuindo também as alucinações comuns aos sintomas psicóticos. A partir deste fármaco, modificações químicas levaram ao desenvolvimento da clorpromazina, que foi considerado o primeiro fármaco antipsicótico (BLIN, 1999), inicialmente classificado como fármaco "Neuroléptico", ou "Tranquilizante Maior" (em oposição aos benzodiazepínicos, os "Tranquilizantes Menores" (KING et al., 2002). É importante ressaltar que tais medicamentos tratam apenas os sintomas e não apresentam perspectivas de cura da doença mental. Além disso, a eficácia clínica atinge cerca de 70% dos pacientes psicóticos, sendo os 30% restantes classificados como "resistentes ou refratários ao tratamento", representando um importante problema na psiquiatria (RANG et al., 2004).

Atualmente, os antipsicóticos classificam-se em típicos e atípicos, seguindo sua respectiva capacidade em desenvolver efeitos adversos extrapiramidais ou não, como também sua potencial eficácia sobre os sintomas negativos da esquizofrenia (BLIN et al., 1999; PROESQ, 2007). Dentre os neurolépticos típicos, estão a clorpromazina, a flufenazina e o haloperidol; já como exemplo de atípicos podem ser citados a clozapina e fármacos de segunda geração, como olanzapina e risperidona.

Os antipsicóticos clássicos ou típicos atuam bloqueando, de modo principal, receptores dopaminérgicos D_2 (CREESE et al., 1976; SNYDER, 1986; FLEISCHHACKER, 2005). Entre os sistemas dopaminérgicos cerebrais nos quais os antipsicóticos atuam, o mesocortical e o mesolímbico são os que provavelmente estão mais relacionados com a fisiopatologia da esquizofrenia. Entretanto, existe uma via dopaminérgica que se projeta da substância negra para o estriado dorsal (caudato-putamen, nos núcleos da base), sendo integrante do "sistema extrapiramidal" (HEIMER, 2003). Os antipsicóticos típicos, ao antagonizarem essa via, induzem os efeitos colaterais chamados "efeitos extrapiramidais", que são alterações motoras agudas e crônicas, como bradicinesia e acatisia, constituindo o reconhecido "Parkinsonismo" e a síndrome da discinesia tardia (DT).

Em relação aos neurolépticos atípicos, a maioria desses fármacos possui a capacidade de bloquear tanto os receptores da serotonina (5-HT) quanto os dopaminérgicos. No entanto, foi observado que, em contraste aos antipsicóticos típicos, eles não foram efetivos em modelos de estereotipia induzidos por anfetamina e apomorfina (HIPPIUS, 1989; HEALY, 2002). Posteriormente, foi observado que pacientes acometidos por sintomas negativos da esquizofrenia passaram a responder de maneira mais satisfatória ao tratamento com os

atípicos (KANE et al., 1988; ROSENHECK et al., 1999; BUCKLEY; STHAL, 2007). Neste sentido, novos antipsicóticos atípicos passaram a ser sintetizados visando minimizar os efeitos colaterais extrapiramidais. Por outro lado, apesar dos atípicos serem menos neurotóxicos, eles são considerados disruptores metabólicos, implicados como causa de intolerância à glicose, desenvolvimento de *diabetes melittus*, pancreatite, ganho de peso e, além disso, podem desencadear discrasias sanguíneas graves, necessitando monitoramento sérico constante (HENDERSON, 2000; MORGAN et al., 2003). Adicionalmente, estes fármacos podem apresentar uma menor eficácia em relação aos sintomas positivos da esquizofrenia e também um maior custo, o que limita o uso por parte da população que apresenta um nível socioeconômico inferior, já que a utilização do medicamento geralmente é em longo prazo, podendo até mesmo ser para a vida toda (SHIRAKAWA et al., 2001). Neste sentido, os neurolépticos típicos, em especial o haloperidol, continuam sendo amplamente empregados no tratamento farmacológico da esquizofrenia (SHARM et al., 2003; PONTO et al., 2010).

1.3 Haloperidol

1.3.1 Aspectos gerais

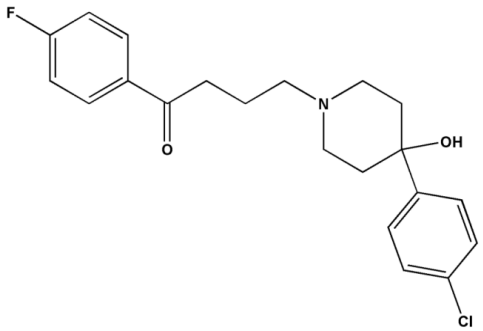

Figura 1 - Estrutura química do haloperidol
Fonte: adaptado de Moor et al. (1992)

O haloperidol, 4-[4-(4-clorofenil)-4-hidroxi-1-piperidinil]-1-(4-fluorofenil)-1-butanona (Figura 1), é um neuroléptico pertencente à classe dos antipsicóticos clássicos ou típicos, que se destaca por sua potência, especificidade e longa duração de ação (NIEMEGEERS, 1983; FROTA, 2001). O medicamento foi a primeira butirofenona a ser descoberta, em 1958, pelo médico e farmacologista belga Paul Janssen, na tentativa de desenvolver um fármaco analgésico opióide (JANSSEN, 1998). Contudo, havia sido

descoberto um fármaco com ação antipsicótica, marcando um acontecimento decisivo na história dos neurolépticos, tornando-se o medicamento para controle de psicoses mais vendido mundialmente (DENIKER, 1998). Enquanto a segunda geração de antipsicóticos apresenta um elevado aumento no uso, particularmente na América do Norte e na Europa, o haloperidol continua sendo um fármaco muito importante no resto do mundo, principalmente em países subdesenvolvidos, devido a sua ampla disponibilidade e baixo custo de aquisição (EMSLEY et al., 1999; PONTO et al., 2010).

O haloperidol é um fármaco eficaz para o controle dos sintomas positivos da esquizofrenia (TIRONE et al., 1985; GLICK et al., 2001), transtornos afetivos, delírios, alucinações, confusão mental e psicoses agudas e crônicas que apresentem agitações psicomotoras (MENEGATTI et al., 2004; GRANGER; ALBU, 2005). Sua ação farmacológica envolve o bloqueio dos receptores dopaminérgicos D_2 na via mesolímibica (CREESE et al., 1976; ANANTH et al., 2004). Além disso, é um potente antagonista dos receptores opióides σ e apresenta fraca atividade antagonista em receptores muscarínicos, histaminérgicos H_1, α-adrenérgicos e serotoninérgicos (DOLLERY, 1991).

O haloperidol encontra-se disponível no mercado na forma de comprimidos e gotas para adminitração oral e na forma injetável, para administração intramuscular. Após administração por via intramuscular, o pico das concentrações plasmáticas do fármaco ocorre dentro de 15 a 60 minutos e a biodisponibilidade é de aproximadamente 75% (JAVAID, 1994). Já por via oral, as concentrações plasmáticas máximas ocorrem geralmente dentro de 3-6 horas (KUNKA; PEREL, 1989) e a biodisponibilidade cai para 65% (HOLLEY et al., 1983). O fármaco se liga fortemente às proteínas plasmáticas, na faixa de 92% (FORSMAN; OHMAN 1976) e é extensivamente metabolizado pelo fígado (FORSMAN et al., 1977). Várias são as vias de biotransformação do fármaco, o qual pode sofrer glicuronidação, redução, oxidação, desalquilação e desidratação, estando essas ilustradas na Figura 2. Dentre os metabólitos formados, destaca-se o haloperidol piridinium (HPP^+), reconhecido por sua hepatotoxicidade (PARIKH et al. 2003; SILVEIRA, 2007) e neurotoxicidade (BLOOMQUIST et al., 1993; 1994; KALGUTKAR et al. 2003). A meia vida do fármaco é de cerca de 20 horas, podendo esta ser muito variável (intervalo de 10-36 horas) (FORSMAN; OHMAN, 1976; HOLLEY et al., 1983) e a excreção é predominantemente urinária (BERESFORD; WARD, 1987).

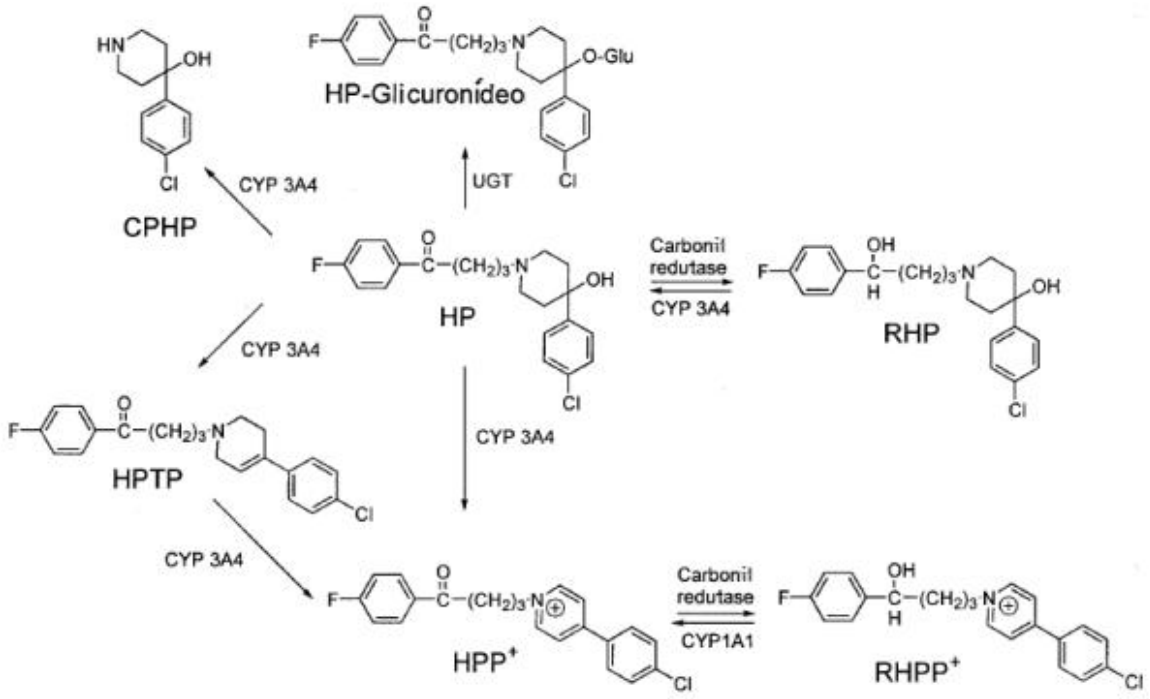

Figura 2 - Vias de biotransformação do haloperidol em humanos
Fonte: adaptado de Kalgutkar et al. (2003)

1.3.2 Efeitos colaterais

Os principais efeitos adversos do haloperidol são os efeitos motores extrapiramidais, como distonia aguda, rigidez muscular, tremores e acatisia. Além disso, outros sintomas também surgem após o uso do fármaco e consistem em agitação, sonolência, insônia, cefaleias, náuseas, visão turva, retenção urinária, hipotermia, hipotensão, depressão, estados confusionais, comprometimento da função sexual, sensibilidade à luz solar, ataques epilépticos, hiperprolactinemia, arritmias ventriculares e anormalidades em testes de função hepática. A DT e a síndrome neuroléptica maligna (reação ao uso de substâncias relacionadas à DA, cujos sintomas principais são rigidez muscular e hipertermia) também foram associadas à terapia com haloperidol (ALLEN et al., 1998) e constituem situações de elevada gravidade. Uma observação importante é que o haloperidol apresenta uma estreita janela terapêutica (DARBY et al., 1995; KUDO; ISHIZAKI, 1999), conduzindo para a necessidade do ajuste das dosagens e monitoramento constante durante o tratamento.

1.3.2.1 Efeitos motores extrapiramidais

Em animais de laboratório, foi observado que o haloperidol e seus metabólitos produzem movimentos anormais como, por exemplo, discinesia orofacial (DO) e comportamento cataléptico (NAIDU et al., 2003; COLPO et al., 2007; BARCELOS et al., 2010; TREVIZOL et al., 2011), os quais têm sido associados a alterações morfológicas nas regiões cerebrais dos núcleos da base, como o corpo estriado e a região da substância negra (MESHUL; TAN, 1994; BARCELOS et al., 2010; 2011).

Em relação à clínica, embora considerado seguro, o uso do haloperidol pode desencadear uma série de efeitos secundários extrapiramidais, incluindo parkinsonismo e DT (CREESE et al., 1976; ALVAREZ; SKOWRONSKI, 2003; ANANTH et al., 2004; TADA et al., 2004; YEN et al., 2004; GRANGER; ALBU, 2005), que pode surgir durante o uso prolongado de doses elevadas do fármaco. Foi verificado que há uma janela terapêutica para a ocupação dos receptores D_2, com resposta antipsicótica, iniciando por volta de 60% de ocupação, enquanto os efeitos extrapiramidais são desencadeados com cerca de 80% de ocupação (KAPUR et al., 2000).

As primeiras descrições da DT foram publicadas entre 1956 e 1957, sendo inicialmente denominada de "discinesia persistente", "síndrome bucolínguo-mastigatória" ou "síndrome da insuficiência extrapiramidal terminal" (KANE, 1995). A DT é uma das principais preocupações geradas pelo tratamento crônico de pacientes esquizofrênicos com antipsicóticos típicos, aparecendo meses ou anos após o início do tratamento, podendo persistir com a retirada do fármaco (ANDREASSEN; JORGENSEN, 1994). Esta síndrome acomete cerca de 20-40% dos indivíduos tratados com haloperidol e demais antipsicóticos típicos (KANE et al.,1986; WOERNER et al., 1998) e menos frequentemente após o tratamento com antipsicóticos atípicos (CHOUINARD, 1995; BEASLEY et al., 1997; GLAZER, 2000). A taxa de casos aumenta com o avanço da idade e uma prevalência superior a 50% tem sido relatada em pacientes mais velhos, com idade acima de 50 anos (YASSA; JESTE, 1992).

A síndrome da DT caracteriza-se por movimentos anormais hipercinéticos, sem propósito, repetitivos e involuntários, os quais podem ocorrer durante ou após uma interrupção do tratamento prolongado com neurolépticos. Estes distúrbios do movimento ocorrem mais frequentemente na região orofacial e compreendem movimentos de mastigação, protrusão da língua, estalido dos lábios, atos de franzir do rosto e piscar de olhos. Em alguns casos, os distúrbios hipercinéticos podem também atingir o pescoço, os membros

(principalmente os superiores) e o tronco (KANE, 1995). Também podem desenvolver sintomas axiais de movimentos pélvicos para frente e para trás ou movimentos rotatórios, descontínuos, dos quadris (LAPORTA et al., 1990).

Algumas hipóteses neuroquímicas têm sido propostas para o desenvolvimento da DT durante as últimas décadas, incluindo a hipersensibilidade dos receptores dopaminérgicos (CASEY, 1994), o desbalanço entre os receptores D_1/D_2 (IKEDA et al., 1999), a perturbação de equilíbrio entre os sistemas dopaminérgico e colinérgico (GERLACH & THORSEN, 1976), a disfunção de neurônios gabaérgico nigro-estriatais (FIBIGER & LLOYD, 1984) e a excitotoxicidade (EBADI & SRINIVASAN, 1995; ANDREASEN & JORGENSEN, 2000). No entanto, os mecanismos moleculares responsáveis pela neurofisiopatofisiologia da DT ainda não foram completamente elucidados.

A supersensibilidade dopaminérgica consiste na hipótese mais popular para explicar o desenvolvimento da DT. Segundo esta hipótese, a síndrome resulta de uma resposta cerebral, secundária ao bloqueio crônico dos receptores dopaminérgicos pelos antipsicóticos, em locais relacionados ao controle dos movimentos, como a via nigro-estriatal. Em resposta a este bloqueio crônico, há um aumento compensatório no número de receptores dopaminérgicos, receptores esses que, provavelmente, respondem a menores níveis de DA, levando a um estado hiperdopaminérgico e a manifestações clínicas como, por exemplo, a DT (KLAWANS; RUBOVITS, 1972; WU et al., 2002).

O aumento na densidade dos receptores D_2 na região estriatal de seres humanos e em animais coincide com o aparecimento dos efeitos secundários extrapiramidais da DT (KLAWANS & RUBOVITS, 1972). No entanto, esta teoria possui algumas contradições: o fator de risco mais importante para o desenvolvimento da DT é a idade (KANE, 1995; WOERNER et al., 1998); contudo, foi demonstrado que o envelhecimento faz com que ocorra a redução do número e da sensibilidade dos receptores dopaminérgicos; também há o fato de que a retirada do fármaco coincide com o retorno da densidade normal dos receptores dopaminérgicos estriatais; porém, a síndrome pode manifestar-se de forma irreversível (SACHDEV, 2000).

Assim, uma hipótese que vem ganhando atenção na literatura é a de que os radicais livres (RL) podem ter uma importante participação no desenvolvimento da DT (CADET et al., 1987; LOHR et al., 2003). Devido ao fato de bloquearem receptores dopaminérgicos pré-sinápticos inibitórios, os antipsicóticos podem causar uma elevação secundária da síntese e liberação de DA, a qual fica vulnerável ao metabolismo oxidativo e à auto-oxidação (LOHR, 1991; ANDREASSEN; JORGENSEN, 2000; ZHU, 2004). A DA, ao sofrer oxidação pela

monoaminoxidase (MAO), gera o ácido 3,4-dihidroxifenilacético (DOPAC) e sabe-se que a atividade de oxidases como a MAO produz o peróxido de hidrogênio (H_2O_2) que, ao reagir com metais de transição, forma RL via reação de Fenton. Além disso, a auto-oxidação da DA forma dopamino-quinonas, que também contribuem na geração de RL (LOHR et al., 1991; 2003; ASANUMA et al., 2003). De fato, a geração de RL e o aumento dos produtos de lipoperoxidação (LPO) podem resultar em estresse oxidativo (EO) em diferentes regiões cerebrais dopaminérgicas (BECHELLI, 2000; BURGER et al., 2005b; BARCELOS et al., 2010; 2011), levando à morte celular (Figura 3). Ainda, o bloqueio dos receptores dopaminérgicos estriatais pode causar excitotoxicidade, através do aumento do glutamato extracelular, elevando assim os danos oxidativos (COYLE; PUTTFARCKEN, 1993; TSAI et al., 1998; BURGER et al., 2005a; ALBENSI, 2007; ZHURAVLIOVA et al., 2007). Adicionalmente, cabe ressaltar o papel tóxico do HPP^+, metabólito que inibe o complexo I da cadeia respiratória, induzindo EO cerebral. Também foi demonstrado que este metabólito é capaz de interagir com os transportadores de aminas biogênicas, induzindo à liberação dessas aminas, bem como o bloqueio da recaptação de DA em sinaptossomas de camundongos (BLOOMQUIST et al., 1993; KOU et al., 2006). O bloqueio da recaptação e o aumento da liberação poderiam exacerbar os níveis sinápticos de DA, contribuindo para a geração de RL e consequente neurotoxicidade (FILLOUX; TOWNSEND, 1993; CAMARERO et al., 2002; ASANUMA et al., 2003).

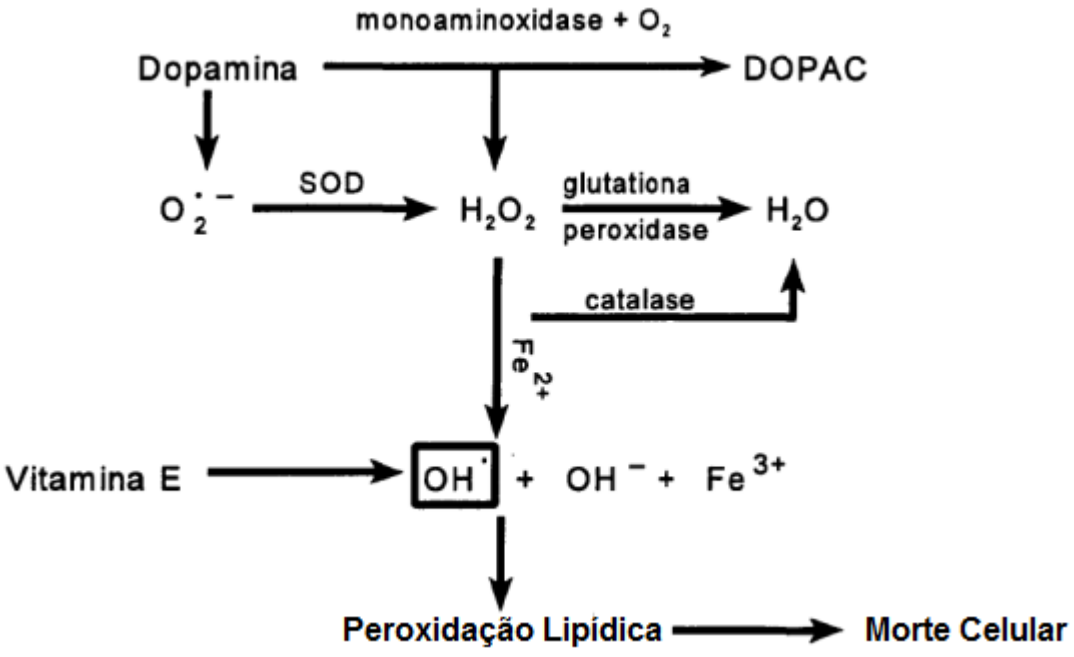

Figura 3 - Oxidação da dopamina e a produção de radicais livres
Fonte: adaptado de Elkashef e Wyatt (1999).

Os níveis de LPO no sangue e líquido cefalorraquidiano de pacientes que sofrem de DT são significativamente maiores, quando comparados a pacientes normais (LOHR et al., 2003; RUKMINI et al., 2004; SARANDOL et al., 2007; ARINOLA; IDONIJE, 2009; HUANG et al. 2010). Além disso, o uso crônico de neurolépticos demonstrou causar diminuição na atividade de enzimas antioxidantes, tais como superóxido dismutase e catalase (CADET et al., 1987; PARIKH et al., 2003; MILJEVIĆ et al., 2010). Ainda, distúrbios do movimento induzidos pelo haloperidol em modelos animais confirmaram a ação protetora de substâncias antioxidantes (BURGER et al., 2005b; COLPO et al., 2007; BARCELOS et al. 2010, TREVIZOL et al., 2011; MAXIA et al., 2012), enquanto o envelhecimento e a neurotoxina mitocondrial do ácido 3-nitropropiônico, os quais elevam a geração de RL, agravaram a DO induzida por haloperidol (CALVENTE et al., 2002).

1.4 Nanotecnologia

1.4.1 Considerações iniciais

A nanotecnologia é definida pela *National Science Foundation* (EUA) como sendo o desenvolvimento de pesquisa e tecnologia nos níveis atômico, molecular ou macromolecular na faixa de dimensões entre 1 e 100 nm (NSF, 2000). Entretanto, em alguns casos particulares, a dimensão crítica para a obtenção de novas propriedades e fenômenos pode se concentrar abaixo de 1 nm (manipulação de átomos) ou acima de 100 nm, como no caso das nanopartículas estudadas na área das ciências da vida, incluindo a área farmacêutica (FRONZA et al., 2007; HIA; NASIR, 2011).

A nanotecnologia foi desenvolvida para contribuir com diferentes ramificações da ciência, com destaque para aplicação nas ciências biomédicas (LIN et al., 2010). Recentemente, "nanotecnologia" tornou-se um termo popular que representa os principais esforços da ciência e da tecnologia atual. Como ela ainda não é uma tecnologia madura é, portanto, mais apropriadamente chamada de nanociência (PARK, 2007). A nanociência, por sua vez, fornece um entendimento fundamental dos fenômenos e materiais em nanoescala, criando e usando estruturas, dispositivos e sistemas que tenham novas propriedades e funções devido ao seu tamanho reduzido (FRONZA et al., 2007). Desta forma, a nanociência representa não apenas uma área específica, mas uma grande variedade de disciplinas que inicia a partir da ciência dos materiais básicos para aplicações em cuidados pessoais (PARK, 2007). Assim, foi verificada uma correlação entre a ascensão de nanomateriais e avanços nas

mais diversas disciplinas, destacando-se engenharia, física, química, robótica, biologia e medicina (FARAJI; WIPF, 2009; MU; SPRANDO, 2010).

Uma das áreas importantes da nanotecnologia é a "nanomedicina" que, de acordo com o *National Institute of Health* (NIH), refere-se à intervenção médica, em escala nanométrica, altamente específica, destinada à prevenção, diagnóstico, monitoramento e tratamento de doenças (MOGHIMI et al., 2005).

O controle da liberação de fármacos em sítios de ação específicos, através da utilização de vetores, capazes de permitir a otimização da velocidade de cedência e do regime de dosagem das substâncias, tem sido uma área de intensa pesquisa (SCHAFFAZICK et al., 2003; BERKLAND et al., 2004). O fármaco vetorizado é definido como uma substância que tem uma liberação seletiva para sítios fisiológicos específicos, órgãos, tecidos ou células, nos quais a atividade farmacológica é requerida (YOKOYAMA; OKANO, 1996; SOPPIMATH et al., 2001). Assim, um aumento na concentração do fármaco em sítios específicos e/ou redução da toxicidade em sítios não específicos pode levar a índices terapêuticos mais adequados (YOKOYAMA; OKANO, 1996).

Tendo em vista a vetorização de fármacos, a ciência farmacêutica passou a aplicar a nanotecnologia nos anos 70, dando início a uma revolução na administração de medicamentos no organismo (SAKATA et al., 2007). Com o auxílio desta tecnologia, um fármaco pode ser incorporado a um sistema nanoestruturado e ser capaz de atingir tecidos infectados e inflamados, os quais jamais seriam atingidos por meio de medicamentos convencionais (BRANDÃO, 2005).

No que diz respeito à liberação de fármacos, é inútil descrever nanotecnologia com base em um limite de tamanho, pois a eficiência e a utilidade dos sistemas de liberação de fármacos não são baseadas apenas em seus tamanhos. Assim, os nanocarreadores de fármacos podem variar de nanosistemas verdadeiros (por exemplo, conjugados de polímero-fármaco) até micropartículas na faixa de 100 µm, pois tanto os sistemas em escala nano quanto micro estão sendo extremamente importantes para o desenvolvimento de vários sistemas de liberação de fármacos utilizados clinicamente. Desta forma, por razões práticas, o termo "nanotecnologia" inclui "microtecnologia" e "nanofabricação" (PARK et al., 2007).

1.4.2 Sistemas carreadores nanoestruturados

Os nanosistemas de liberação de fármacos constituem uma parcela significativa da nanomedicina (PARK et al., 2007; DANHIER et al., 2010; CHACKO et al., 2011;

VERCAUTEREN et al., 2012). A nanotecnologia farmacêutica iniciou a partir da década de 70, com o surgimento dos lipossomas. Em seguida, foram desenvolvidos os sistemas nanoparticulados, empregando polímeros biodegradáveis sintéticos (COUVREUR et al., 1979). Já nos anos 90, surgiram as nanopartículas lipídicas sólidas e os nanosistemas mais sofisticados, denominados sistemas furtivos, os quais apresentam revestimentos contendo polímeros hidrofílicos, que permitem um aumento no tempo de circulação no organismo (BARRAT et al., 2000).

A estrutura, o tamanho e a forma dos nanosistemas fornece uma nova dimensão de controle físico, podendo ocorrer uma adaptação específica para determinada função (FARAJI et al., 2009). Neste sentido, utilizando-se sistemas de liberação em nanoescala, as propriedades farmacológicas de determinada substância podem ser drasticamente aprimoradas, levando à descoberta de candidatos farmacológicos seguros e eficazes (WAGNER et al., 2006; DAVIS et al., 2008). Desta forma, compostos que sofrem alguns impedimentos, os quais dificultam o desenvolvimento de medicamentos, como baixa solubilidade em água, toxicidade ou baixa biodisponibilidade, podem ter suas características melhoradas com o auxílio de um nanocarreador (FORREST & KWON, 2008).

Pelo fato de apresentarem elevada área superficial, os sistemas nanoestruturados podem ser considerados vetores para administração de diversas substâncias, principalmente as lipofílicas (GUTERRES et al., 2007) e podem ser administrados pelas vias intravenosa, subcutânea, intramuscular, ocular, oral e tópica (COUVREUR et al., 2002).

Os nanocarreadores fornecem inúmeras vantagens, quando comparados à liberação convencional de fármacos. Como já mencionado acima, estes sistemas podem modificar as propriedades das substâncias, melhorando a solubilidade de compostos hidrofóbicos, tornando-os adequados para a administração parenteral (CHEN et al., 2011a). Além disso, eles aumentam a estabilidade de uma variedade de agentes terapêuticos, como peptídeos e oligonucleotídeos (KOO et al., 2005). E, de modo especial, por serem capazes de alterar parâmetros farmacocinéticos, podem melhorar a biodistribuição (conduzindo a uma vetorização das substâncias até órgãos, tecidos ou células específicas, por meio de um aumento da permeabilidade e efeito de retenção), prolongar a meia-vida dos fármacos, aumentar a biodisponibilidade e possibilitar uma liberação homogênea, controlada e sustentada (HILLERY et al., 2001; VERMA; GARG, 2001; SURI et al., 2007; FARAJI et al, 2009). Consequentemente, os efeitos adversos sistêmicos podem ser reduzidos, bem como a dose e a frequência de administração do fármaco, fazendo com que aumente a aderência ao tratamento por parte do paciente (MATSUMURA; MAEDA, 1986; TAO; DESAI, 2001;

ALLEN; CULLIS, 2004). Além disso, a possibilidade de administração pela via parenteral é capaz de prevenir a degradação gastrintestinal dos fármacos (LAVELLE et al., 1995; SAHOO; LABHASETWAR, 2003). Outra vantagem é que os nanosistemas podem ser utilizados para liberação de fármacos no SNC, devido ao seu tamanho reduzido e maior permeabilidade, através da BHE (KREUTER et al., 2001; BERNARDI et al., 2009; FROZZA et al., 2010). Por fim, eles são capazes de evitar reações de hipersensibilidade e proporcionam uma boa compatibilidade aos tecidos, visto que são fabricados com materiais biodegradáveis (LOBENBERG, 2003).

Sistemas nanoestruturados como carreadores farmacológicos têm sido propostos, principalmente para agentes antitumorais, peptídeos, proteínas, vacinas e agentes anti-infecciosos (COUVREUR; VAUTHIER, 2006). Os principais sistemas coloidais incluem as nanoemulsões, nanoesferas, nanocápsulas, lipossomas e complexos lipídicos (GARCIA-GARCIA et al., 2005). Na Figura 4, estão representados alguns exemplos de nanosistemas que podem ser utilizados como carreadores farmacológicos.

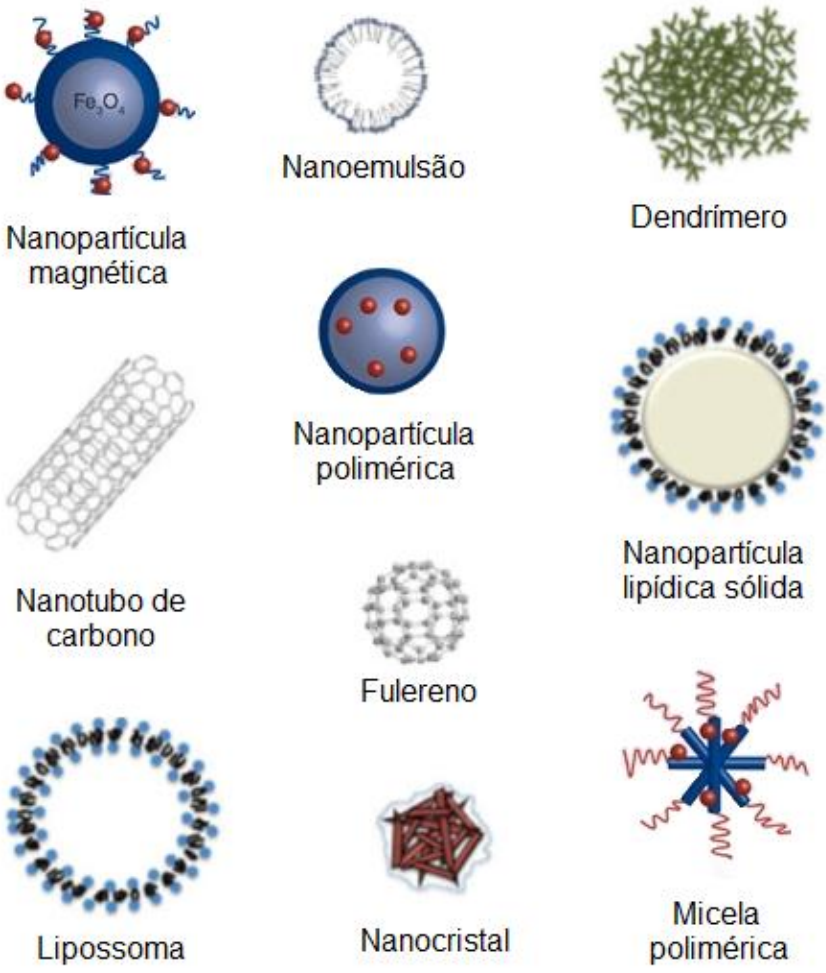

Figura 4 - Representação de alguns sistemas nanocarreadores farmacológicos
Fonte: adaptado de Faraji et al. (2009) e Muthu e Singh (2009)

1.4.2.1 Nanopartículas poliméricas

Há cerca de vinte anos formulações com nanopartículas poliméricas têm sido desenvolvidas como alternativa ao uso de formulações contendo fármacos livres. As nanopartículas poliméricas são sistemas carreadores de fármacos que apresentam diâmetro inferior a 1 µm. Dependendo do processo de preparação das nanopartículas, podem-se obter tipos de estruturas diferentes, as nanoesferas e as nanocápsulas, as quais diferem entre si segundo a composição e organização estrutural (Figura 5) (SOPPIMATH et al., 2001; SCHAFFAZICK et al., 2003; DURÁN et al., 2006; SANTOS; FIALHO, 2007; OLIVEIRA, 2009; MELO, 2010). As nanocápsulas são sistemas vesiculares constituídos por um invólucro polimérico, disposto ao redor de um núcleo lipofílico, podendo o fármaco estar disperso ou dissolvido nesse núcleo e/ou adsorvido à parede polimérica. Dessa forma, as nanocápsulas podem ser consideradas um sistema "reservatório" (COUVREUR et al., 2002). Por outro lado, as nanoesferas, denominadas sistemas matriciais, não apresentam óleo em sua composição, sendo formadas apenas por uma matriz polimérica, a qual retém e/ou adsorve o fármaco (COUVREUR et al., 1995; SCHAFFAZICK et al., 2003; GUTERRES et al., 2007).

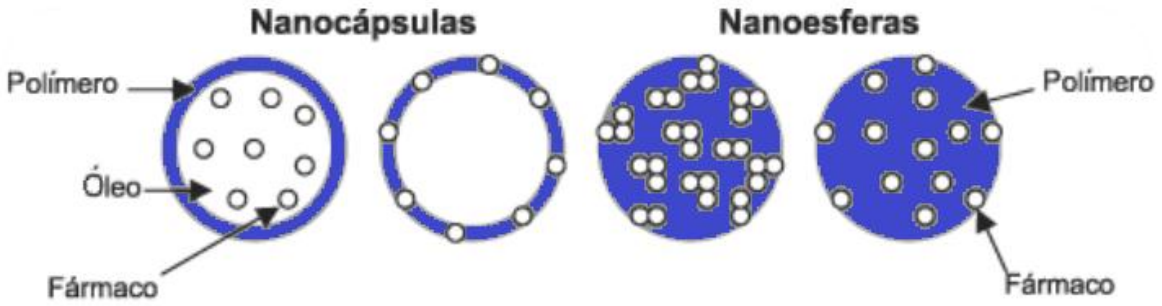

Figura 5 - Representação esquemática dos sistemas de nanopartículas poliméricas
Fonte: adaptado de Schaffazick et al. (2003)

Diversos são os métodos descritos na literatura para a preparação de nanopartículas poliméricas, destacando-se a técnica de nanoprecipitação descrita por Fessi et al. (1989), a qual envolve a deposição interfacial de polímeros pré-formados. O método consiste na dissolução do polímero em uma fase orgânica contendo o fármaco e posterior precipitação, quando vertido sob uma fase aquosa, contendo um tensoativo hidrofílico. Esta técnica origina as nanoesferas, com diâmetros médios situados entre 200 e 500 nm. Entretanto, se incorporado um óleo juntamente a fase orgânica, serão originadas as nanocápsulas, as quais

objetivam uma maior eficiência de incorporação de fármacos lipofílicos. O óleo comumente utilizado para a preparação de nanocápsulas é formado por uma mistura de triglicerídeos de cadeia média (TCM) (DHANIKULA et al., 2007).

As nanocápsulas apresentam uma série de vantagens em relação a outros sistemas carreadores e emulsões, pois conferem proteção da substância ativa nela incorporada frente à degradação química, uma vez que a substância ativa fica retida no interior de uma matriz sólida ou de um invólucro polimérico que também tem a função de controlar a liberação da substância ativa ao meio de dispersão (DINGLER et al., 1999; LAMBERT et al., 2000; COUVREUR et al, 2002; VRIGNAUD et al., 2011).

Inicialmente, as nanopartículas apresentavam a limitação de permanecerem por menos tempo na circulação sanguínea, por serem capturadas pelo sistema fagocitário mononuclear. Como regra geral, a opsonização de partículas hidrofóbicas, em comparação com as partículas hidrofílicas, ocorre mais rapidamente, devido à adsorção das proteínas plasmáticas nestas superfícies (MULLER et al, 1992.; NORMAN et al., 1992). Posteriormente, foram desenvolvidas nanopartículas capazes de vencer a fagocitose, as quais permanecem por um tempo maior na circulação, sendo, portanto, denominadas, “invisíveis” (BRIGGER et al., 2002). Neste sentido, um método amplamente usado para opsonização lenta é o uso de grupos de blindagem adsorvidos ou enxertados à superfície das nanopartículas, bloqueando interações eletrostáticas que facilitam a ligação das opsoninas a essa superfície. Esses grupos tendem a ser longas cadeias de polímeros hidrofílicos, como o polietilenoglicol (PEG) e tensoativos não-iônicos, como os polisorbatos (PERACCHIA et al., 2003).

O tamanho e a distribuição de tamanho das nanopartículas são importantes para determinar a interação dessas com a membrana celular, assim como a penetração através das barreiras fisiológicas. O tamanho necessário para as nanopartículas atravessarem diferentes barreiras biológicas é dependente do tecido, do sítio alvo e da circulação (BRANNON-PEPPAS; BLANCHETTE, 2004). Já a carga da superfície das nanopartículas também é importante, visto que pode determinar se as partículas tenderiam a se agrupar no fluxo sanguíneo, se aderir ou interagir com cargas opostas nas membranas das células (FENG, 2004).

Os polímeros biodegradáveis estão entre os materiais que têm sido extensivamente utilizados na preparação de nanopartículas, principalmente por não apresentarem toxicidade ao corpo humano, sendo substâncias facilmente metabolizadas (HASIRCI et al., 2000). Estes polímeros formam uma rede, a qual propicia uma melhor retenção do fármaco, permitindo

uma degradação mais lenta e, consequentemente, uma liberação prolongada do ativo (MULLER et al., 2000).

Dentre os polímeros utilizados, destaca-se a poli(ε-caprolactona) (PCL), cuja propriedade de biodegradabilidade foi identificada pela primeira vez em 1973 (MURTHY, 1997). Devido à natureza biocompatível e biodegradável, a PCL tem sido amplamente estudada em formulações que visam um controle de liberação do fármaco. Sua elevada permeabilidade a uma gama de medicamentos permite a distribuição uniforme dos fármacos na matriz polimérica e, por ser um polímero semicristalino, apresenta uma degradação mais lenta, o que facilita a liberação sustentada do fármaco (GUTERRES et al., 2007), até mesmo por vários meses (HAKKARAINEN; ALBERTSSON, 2002; SINHA et al., 2004). Sua natureza não-tóxica e sua citocompatibilidade com uma série de tecidos corporais o torna um material ideal para engenharia tecidual. Além disso, é utilizado na medicina, em curativos, suturas, contraceptivos e na odontologia (LU & CHEN, 2004), devido às suas propriedades mecânicas, como boa flexibilidade, elasticidade e resistência à tração (ESTELLE et al., 2008; WOODRUFF; HUTMACHER, 2010).

Os sistemas contendo nanopartículas poliméricas têm sido desenvolvidos visando inúmeras aplicações terapêuticas, sendo planejados, principalmente, para administração parenteral, oral, oftálmica ou tópica (SCHAFFAZICK et al., 2003). Vários medicamentos, das mais diversas classes farmacológicas, mostraram-se capazes de serem nanoparticulados, tais como antibióticos (MOHAMMADI et al., 2010), anti-inflamatórios (MARCHIORI et al., 2010), antivirais (MAINARDES et al., 2010), antifúngicos (CHEN et al., 2008), antitumorais (MATTHEOLABAKIS et al., 2009), entre outros. Além disso, nanopartículas poliméricas destinadas ao controle de doenças severas, como câncer (MU; FENG, 2003), AIDS (COESTER et al., 2000), diabetes (DAMGE et al., 2007), malária (DATE & JOSHI, 2007) e tuberculose (AHMAD et al., 2006) estão em diferentes fases de testes e algumas já estão sendo comercializadas (KIM;LEE, 2001; LEE et al., 2008).

Assim como os demais sistemas nanoestruturados, as nanopartículas poliméricas biodegradáveis são frequentemente utilizadas para proteger moléculas lábeis (FLORES et al., 2011; OURIQUE et al., 2011) ou aquelas que sofrem degradação enzimática ou imunológica (HAN et al., 2009) e que apresentam baixa biodisponibilidade e baixa solubilidade em água (WU et al., 2008; MAULUDIN et al. 2009a; 2009b), aumentando, desta forma, a biodisponibilidade e o tempo de retenção. Estas formulações também vetorizam o fármaco (NAGARWAL et al., 2009; ZHU et al., 2009), aumentando a especificidade e reduzindo os efeitos adversos sistêmicos (BECK et al., 2005; YEN et al., 2008; GAO et al., 2010). Além

disso, promovem uma liberação controlada e sustentada do ativo (BECK et al., 2005; 2006; 2007; FONTANA et al., 2009; MARCHIORI et al., 2010) e aumentam a eficácia farmacológica (SCHAFFAZICK et al., 2008; BERNARDI et al., 2009; OURIQUE et al., 2010; FONTANA et al., 2011; IANISKI et al., 2012), possibilitando uma redução da dose e da frequência de administração do fármaco.

1.4.2.1.1 Nanocápsulas contendo diferentes núcleos oleosos

O tipo de fase oleosa usada como núcleo na preparação de nanocápsulas poliméricas pode modificar as propriedades de uma formulação, pelo fato de influenciar no tamanho médio de partícula, no índice de polidispersão e na estabilidade, devido à diferença de viscosidade, tensão interfacial e características hidrofóbicas (SCHAFFAZICK et al., 2003; BOUCHEMAL et al., 2004). A diferente composição de ácidos graxos presente no óleo poderia, além disso, apresentar um efeito terapêutico *per se* (ALMEIDA et al., 2009; 2010), modificando a resposta biológica. Neste sentido, óleos vegetais estão sendo utilizados na preparação de nanocápsulas poliméricas (DHANIKULA et al., 2007; ALMEIDA et al., 2009; 2010, FLORES et al., 2011; RIGO, 2011). Estudos realizados por Almeida e colaboradores (2009; 2010) demostraram que o óleo de semente de uva mostrou-se compatível e adequado para a preparação de nanocápsulas contendo benzofenona-3, uma substância fotoprotetora e rutina, um flavonóide com propriedades antioxidantes.

O óleo de semente de uva é composto por 90%, em média, de ácidos graxos mono e poliinsaturados, que são responsáveis por seu valor nutritivo como óleo comestível, especialmente, ácido linoléico (n-6), seguido pelo ácido oléico (n-9) e menor quantidade de ácidos graxos saturados (10%) (BOCKISCH, 1993; FIRESTONE, 1999). Além disso, este óleo possui tocoferóis (5-52mg/100g) e numerosos bioflavonóides polifenólicos, conhecidos por apresentar atividades farmacológicas e terapêuticas potenciais (FIRESTONE, 1999) destacando-se o efeito antioxidante (YAMAGUCHI, 1999; SHAFIEE, 2003). E, devido a este potencial antioxidante, vinte vezes superior à vitamina E e cinquenta vezes maior que a vitamina C (SHI et al., 2003), foi classificado pelo FDA como um ingrediente alimentar seguro.

Em relação à indústria farmacêutica, o óleo de semente de uva possui grande potencial nos medicamentos para uso interno que precisam de óleo como veículo (transporte) (PASSOS et al., 2009), visto que a presença de vitamina E protege da degradação outras substâncias que estiverem juntamente inseridas nos medicamentos (MIGUEL, 1983).

Além do óleo de semente de uva, outros óleos destacam-se, devido ao potencial terapêutico, como amêndoas doces (AHMAD, 2010), gérmen de trigo (MURSEL et al., 2011), gergelim (REZA et al., 2011), oliva (VISIOLI et al., 2002) e, de modo especial, o óleo de peixe (WANG et al., 2006). O óleo de peixe, encontrado especialmente em peixes marinhos (WAINWRIGHT, 2002), é composto principalmente por ácidos graxos essenciais da série ômega-3, tais como: eicosapentanóico (EPA), docosahexaenóico (DHA), α-linolênico (LA) e docosapentaenóico (DPA) (MANTZIORIS et al., 2000). Estes ácidos graxos são importantes para a integridade anatômica, histológica e bioquímica cerebral (ZARARSIZ et al., 2006), visto que estão relacionados com as mais diversas funções cerebrais, especialmente na modificação da membrana celular, influenciando na sua fluidez, na atividade de enzimas, no número e afinidade de receptores, na função de canais iônicos, na transdução de sinais e fatores de crescimento neuronais e na atividade e afinidade de neurotransmissores, (YEHUDA et al., 2005) principalmente da DA (CHALON et al., 1998; WAINWRIGHT, 2002).

Assim, os ácidos graxos da série ômega-3, por agirem em regiões cerebrais críticas como hipocampo (SARSILMAZ et al., 2003a), corpo estriado (SARSILMAZ et al., 2003b) e hipotálamo (SONGUR et al., 2004), são capazes de conferir proteção em diferentes desordens neurológicas e neuropsiquiátricas (BLACK et al., 1979; 1984; ASSISSI et al., 2006). Contrariamente, sua carência tem sido associada ao desenvolvimento de diferentes psicopatologias, como depressão (SILVERS, et al., 2005), hiperatividade e déficit de atenção (YEHUDA et al., 2005), aceleração do processo de envelhecimento (BOURRE, 2004), doença de Huntington (CLIFFORD et al., 2002) e esquizofrenia (JOY et al. 2000; ARVINDAKSHAN et al., 2003; DAS, 2004; SIVRIOGLU et al., 2007).

No que diz respeito a distúrbios do movimento e déficit cognitivo, o óleo de peixe, ou diretamente os seus ácidos graxos, já mostraram diversos benefícios. Assim, experimentos conduzidos pelo laboratório já mostraram os efeitos protetores do óleo de peixe em modelos animais de DO induzidos por reserpina ou mesmo pelo próprio haloperidol em ratos (BARCELOS et al., 2010; 2011), os quais também foram mostrados em primatas por outros pesquisadores (SAMADI et al., 2006). Na clínica humana, a suplementação de ácidos graxos ômega-3 a pacientes psiquiátricos portadores de DT mostrou efeitos antidiscinéticos (VADDADI et al., 1989; EMSLEY et al., 2002; 2003). Ainda, estudos envolvendo aquisição de memória espacial em animais (CALON et al., 2004; 2005, BARCELOS et al., 2010) e função cognitiva associada ao Alzheimer (HASHIMOTO et al., 2002; 2006) também mostraram os efeitos benéficos dos ácidos graxos da série ômega-3, orientando para o seu uso

como uma defesa nutracêutica de fácil acesso e baixa toxicidade, atuante em distúrbios cerebrais (CALON & COLE, 2007).

1.4.3 Vetorização de fármacos ao Sistema Nervoso Central

1.4.3.1 Barreira hematoencefálica

A BHE representa um grande obstáculo para a passagem de moléculas ativas do compartimento sanguíneo para o cérebro (DAVSON & SEGAL, 1996). Ela é composta por diferentes tipos celulares: células endoteliais, pericitos, astrócitos e microglias (ABBOTT et al., 2010). Uma característica importante é que as células do endotélio microvascular cerebral apresentam uma morfologia distinta, pois exibem junções intercelulares, denominadas *tight junctions* (junções oclusivas), ausência de fenestrações e uma atividade pinocítica diminuída (Figura 6). Além disso, estão contidas, nestas células, uma variedade de enzimas, tanto em nível citosólico quanto em nível de membrana extracelular, que metabolizam vários fármacos. Ainda existem as limitações fisiológicas, tais como alta resistência elétrica, gradiente de carga aniônica/catiônica e a presença de transportadores de efluxo, responsáveis pela manutenção da homeostase cerebral, tais como MDR–proteína resistente a múltiplas drogas, MOAT–transportadores orgânicos aniônicos e a glicoproteína P. (DEEKEN; LOSCHER, 2007). Assim, o somatório de todos estes fatores ajuda a restringir a passagem de substâncias através da BHE (BODOR & BUCHWALD, 1999).

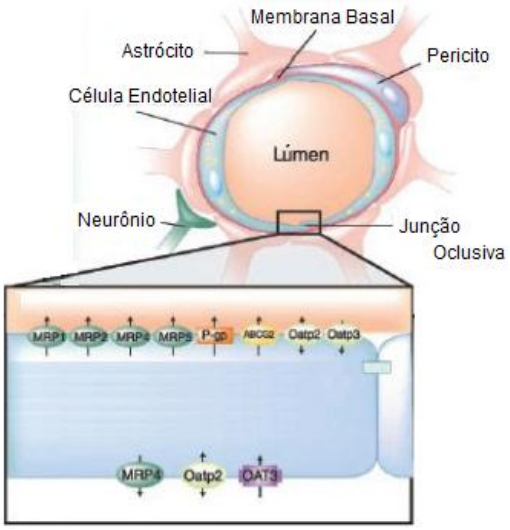

Figura 6 - Anatomia da barreira hematoencefálica
Fonte: adaptado de Deeken e Loscher (2007)

A característica da BHE, de evitar a passagem de moléculas grandes, também enfraquece a eficiência do aporte de fármacos ao cérebro (JEFFREY & SUMMERFIELD, 2010). Para que os fármacos possam atingir o cérebro, de forma passiva, eles devem apresentar algumas propriedades, tais como o tamanho da molécula (menor que 5.000 Daltons), propriedades lipofílicas e carga elétrica neutra. Uma vez dentro do cérebro, estes fármacos devem vencer o sistema de tranportadores, para não serem eliminados para fora do cérebro (GABATHULER, 2010).

1.4.3.2 Sistemas nanocarreadores em nível cerebral

Os nanocarreadores cerebrais apresentam vantagens quando comparados às terapias convencionais, pois além de serem mais eficazes podem ser administrados de forma não-invasiva, melhorando a qualidade de vida dos pacientes. Atualmente, existem várias aplicações importantes para as nanopartículas em nível cerebral. Um importante aspecto seria o carreamento de agentes diagnósticos com a finalidade de gerar imagens. Outra aplicação importante envolve a situação dos tumores cerebrais que apresentam os vasos sanguíneos mais permeáveis em relação aos vasos dos tecidos normais. Além destas, o uso de nanocarreadores para o tratamento de doenças neurodegenerativas (GARCIA-GARCIA et al., 2005) cria novas perspectivas em relação a doenças tidas como incuráveis, como Alzheimer e esclerose múltipla (KREUTER et al., 2002). Além das nanopartículas permitirem o acesso, através da BHE, de fármacos não-transponíveis, mascarando suas características físico-químicas através do encapsulamento nesses sistemas (GARCIA-GARCIA et al., 2005), também podem ser usadas como sistemas de liberação controlada para prolongar a disponibilidade de fármacos que atravessam livremente a BHE; porém, apresentam uma curta duração de ação no SNC (FRIESE et al., 2000).

Várias são as substâncias, com as mais variadas atividades terapêuticas, utilizadas para tratamento de distúrbios em nível de SNC e que, após terem sido testadas, mostraram-se capazes de serem associadas a sistemas nanocarreadores. Dentre estas substâncias estão quimioterápicos (BERNARDI et al., 2009; KUO; LIANG, 2011; WOHLFART et al., 2011), anti-inflamatórios (KIM; MARTIN, 2006; BERNARDI et al., 2010), antibióticos (PANDEY; KHULLER, 2006; XU et al., 2009; WANG et al., 2010a, 2010b), antifúngicos (CHEN et al., 2011b; XU et al., 2011), antivirais (MAHAJAN et al., 2010; WONG et al., 2010), antiparasitários (GUPTA et al., 2007; MIMCHE et al., 2011), antipsicóticos (MUTHU et al., 2008; 2009; PARIHK et al., 2010), ansiolíticos (PRIPREM et al., 2008), fármacos para

doenças neurodegenerativas (HU et al., 2011; MITTAL et al., 2011; WEN et al., 2011; IANISKI et al., 2012), entre outros.

O mecanismo pelo qual as nanopartículas são capazes de atravessar a BHE ainda não está totalmente elucidado. No entanto, existe uma série de possibilidades, as quais poderiam explicar esta penetração: 1) um aumento na retenção das nanopartículas nos capilares sanguíneos cerebrais, criando um gradiente de concentração maior, que facilitaria o transporte através da camada de células endoteliais; 2) um efeito tensoativo, caracterizado por uma solubilização dos lipídeos da membrana das células endoteliais, o que poderia levar à fluidização da membrana e, portanto, aumentar a permeabilidade do fármaco através da BHE; 3) as nanopartículas poderiam ser capazes de proporcionar uma abertura das "tight junctions" entre as células endoteliais, favorecendo a penetração do fármaco na sua forma livre ou em conjunto com as nanopartículas; 4) as nanopartículas poderiam sofrer endocitose pelas células endoteliais e, assim, liberarem o fármaco dentro dessas células, chegando ao cérebro; 5) o sistema nanopartícula-fármaco poderia sofrer transcitose, através da camada de células endoteliais; 6) o polissorbato 80, usado como agente de revestimento, poderia estar inibindo o sistema de efluxo, de modo particular a glicoproteína-P. Além disso, todos estes mecanismos poderiam estar atuando em conjunto (KREUTER et al., 2001; 2002, 2005; GARGIA-GARCIA et al., 2005; WONG et al., 2012).

1.4.3.3 Antipsicóticos associados a nanocarreadores

Até o momento, já foram realizadas diversas pesquisas visando o desenvolvimento de sistemas nanoestruturados contendo fármacos antipsicóticos, como a risperidona (KUMAR et al., 2008; 2009; SINGH; MUTHU, 2007; MUTHU; SINGH, 2008; MUTHU et al.., 2009; SILVA et al., 2011), clozapina (VENKATESWARLU; MANJUNATH, 2004; MANJUNATH; VENKATESWARLU, 2005), sulpirida, (PARIHK et al., 2010); olanzapina (VIVEK et al., 2007; SEJU et al., 2011), tioridazina (LAI et al., 2006) e o próprio haloperidol (BUDHIAN et al., 2005; 2007; 2008).

Estudos de caracterização físico-química destas nanoestruturas mostraram uma modificação no perfil de liberação *in vitro* da risperidona (MUTHU; SINGH, 2008; MUTHU et al., 2009), tioridazina (LAI et al., 2006), olanzapina (VIVEK et al., 2007; SEJU et al., 2011) e haloperidol (BUDHIAN et al., 2008), possibilitando uma liberação mais sustentada e controlada. Além disso, foi verificado *in vivo* um aumento da biodisponibilidade e da concentração plasmática da clozapina (VENKATESWARLU; MANJUNATH, 2004;

MANJUNATH; VENKATESWARLU, 2005) ou uma diminuição da eliminação da sulpirida (PARIHK et al., 2010), quando veículadas a nanosistemas. Ainda, através de estudos de distribuição em modelos animais, foi observado um maior aporte cerebral de olanzapina (SEJU et al., 2011) e clozapina (MANJUNATH; VENKATESWARLU, 2005) quando administradas na forma nanoparticulada do que na forma livre.

No entanto, estudos de eficácia terapêutica e efeitos colaterais de nanopartículas contendo antipsicóticos são escassos na literatura. Um estudo realizado por Muthu et al. (2009) mostrou que nanopartículas poliméricas contendo risperidona apresentaram maior atividade antipsicótica em camundongos, por meio de um modelo de pseudo-psicose induzido por apomorfina, bem como uma redução no comportamento cataléptico.

No que diz respeito ao haloperidol associado a nanopartículas, até o momento de realização do presente trabalho haviam sido realizados estudos mostrando apenas a preparação, a caracterização (BUDHIAN et al., 2005; 2007; DHANIKULA et al., 2007) e a liberação *in vitro* do fármaco (BUDHIAN et al., 2008). Em relação ao desenvolvimento de sistemas de haloperidol nanoencapsulado, já foi observada a substituição do núcleo oleoso composto por TCM, pelos óleos de girassol e óleo de soja (DHANIKULA et al., 2007). No entanto, não há registros científicos de estudos pré-clínicos, demonstrando a eficácia terapêutica ou os efeitos adversos destas formulações de nanocápsulas contendo haloperidol e tampouco o emprego do óleo de peixe e óleo de semente de uva na preparação desses sistemas.

2 OBJETIVOS

2.1 Objetivo geral

Desenvolver suspensões de nanocápsulas de haloperidol contendo diferentes núcleos oleosos e avaliar a atividade antipsicótica e os efeitos colaterais motores, relacionados ao estresse oxidativo, em ratos.

2.2 Objetivos específicos

• Desenvolver suspensões de nanocápsulas de haloperidol contendo triglicerídeos de cadeia média e núcleos oleosos alternativos, compostos por óleo de peixe e óleo de semente de uva.

• Avaliar a eficácia terapêutica do haloperidol nanoencapsulado em ratos e comparar ao fármaco livre.

• Avaliar a incidência e gravidade de efeitos colaterais motores agudos e subcrônicos em ratos, comparando as formulações de haloperidol livre e nanoencapsulado.

• Avaliar marcadores de estresse oxidativo em tecidos neuronais da região extrapiramidal de ratos tratados com haloperidol livre e nanoencapsulado e suas relações com o desenvolvimento de distúrbios do movimento.

• Realizar um estudo comparativo entre três formulações de haloperidol nanoencapsulado contendo diferentes óleos (triglicerídeos de cadeia média, óleo de semente de uva e óleo de peixe) sobre o desenvolvimento de discinesia oral aguda e subcrônica em ratos.

• Avaliar a viabilidade celular e a geração de radicais livres em tecidos da região extrapiramidal de ratos tratados com três formulações de haloperidol nanoencapsulado contendo diferentes óleos (triglicerídeos de cadeia média, óleo de semente de uva e óleo de peixe).

3 MANUSCRITOS CIENTÍFICOS

Os resultados inseridos nesta tese apresentam-se sob a forma de artigos científicos, os quais se encontram aqui estruturados. Os ítens Materiais e Métodos, Resultados, Discussão dos Resultados e Referências Bibliográficas,encontram-se nos próprios artigos, os quais estão dispostos da mesma forma que foram publicados (01), aceitos para publicação (01) e em fase de submissão (01).

3.1 Artigo 1

HALOPERIDOL-LOADED POLYSORBATE-COATED POLYMERIC NANOCAPSULES INCREASE ITS EFFICACY IN THE ANTIPSYCHOTIC TREATMENT IN RATS

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Note

Haloperidol-loaded polysorbate-coated polymeric nanocapsules increase its efficacy in the antipsychotic treatment in rats

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Catalepsy

ABSTRACT

Haloperidol is an antipsychotic drug associated with the development of movement disorders. We evaluated the effect of its nanoencapsulation on its pharmacological activity and motor side effects. Haloperidol-loaded polysorbate-coated nanocapsules (H-NC) showed nanometric size, negative zeta potential and low polydispersity indices and high encapsulation efficiency (>95%). Rats received a single dose of H-NC (0.2 mg/kg ip) and four doses of D,L-amphetamine, AMPH (8.0 mg/kg ip), injected every 3 h (0, 3, 6 and 9 h). The AMPH-induced stereotyped movements were quantified in the intervals of 15 min after each of four doses of AMPH, demonstrating greater pharmacological efficacy of the H-NC over free haloperidol (FH). The acute motor side effects were evaluated 1 h after a single dose of H-NC or its free solution (0.2 mg/kg ip). The group treated with H-NC presented lower extrapyramidal effects (catalepsy and oral dyskinesia) than those treated with FH. In the last experimental set, rats sub-chronically treated with a daily dose of H-NC (0.2 mg/kg ip) for 28 days showed a lower incidence of extrapyramidal effects than those treated with the free drug (0.2 mg/kg ip). Our findings showed the potential of using H-NC in the development of a nanomedicine aimed at increasing the efficacy of this antipsychotic drug and reducing its side effects.

1. Introduction

Haloperidol is an antipsychotic drug that causes movement disorders such as parkinsonism and tardive dyskinesia, which is characterized by involuntary movements frequently irreversible and disabling.

Body weight gain and diabetes development are side effects of the more recent atypical neuroleptics, so that typical neuroleptics such as haloperidol are still the most widely used drugs to treat psychiatric disorders.

Polymeric nanoparticles have attracted attention as drug delivery systems and can be employed to carry and release drugs at controlled rate in specific body sites [1], especially in the central nervous system (CNS). We hypothesized that by loading haloperidol in polysorbate-coated nanocapsules, we could improve its pharmacological efficacy and/or reduce its side effects, proposing its use as a nanomedicine. To the best of our findings, no study has been previously designed to evaluate the improved efficacy of haloperidol by its nanoencapsulation in polymeric nanoparticles.

2. Materials and methods

2.1. Preparation and characterization of nanocapsules suspension

2.1.1. Preparation of nanocapsules suspension

Haloperidol-loaded nanocapsules (H-NC) were prepared by interfacial deposition of preformed polymer [2]. Blank nanocapsules (B-NC) were prepared as controls using the same protocol of H-NC, but omitting the presence of the drug. A free suspension of haloperidol (0.25 mg/mL) was prepared in water using 5% (w/v) of polysorbate 80.

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2.1.2. Physicochemical characterization

Particle sizes, polydispersity indices and zeta potential were measured by photon correlation spectroscopy using Zetasizer® Nano Series equipment (Zetasizer Nanoseries ZEN 3600, Malvern Instruments, UK).

2.1.3. Determination of drug content and encapsulation efficiency

Drug content (mg/mL) was assayed by HPLC (Gemini RP-18 column (150 × 4.60 mm, 5 μm) and a Shimadzu instrument (UV–VIS SPD-10AVP Module). The mobile phase consisted of methanol/potassium phosphate monobasic pH 4.0 (60:40% v/v) and was pumped through the system at a flow rate of 1.0 mL/min. The volume injected was 20 μL, and haloperidol was detected at 254 nm. HPLC assay demonstrated that this method was linear ($r = 0.9979$) in the range of 5–40 μg/mL and precise (RSD: 2.58% for repeatability and 0.63% for intermediate precision). Free drug was determined in the clear supernatant following separation of nanocapsules by a combined ultrafiltration–centrifugation technique. Encapsulation efficiency (%) was calculated by the difference between the total and free drug concentrations determined in the nanocapsule suspension and in the ultrafiltrate, respectively, using the HPLC method described above.

2.2. In vivo experiments

This study was approved by the Animal Ethical Committee (Universidade Federal de Santa Maria-22/2010), which is affiliated to the Brazilian college of animal experimentation (COBEA) in accordance with international rules of ethics in research.

2.2.1. Experiment 1: pseudo-psychosis induced by D,L-amphetamine (AMPH)

AMPH treatment promotes a set of positive symptoms similar to schizophrenia. Five groups of male Wistar rats ($n = 7$) were allocated in mirrored individual cages, allowing the assessment when the animal was facing away from the observer. Considering the abbreviations: AMPH (amphetamine), FH (free haloperidol), H-NC (haloperidol-loaded nanocapsules), B-NC (blank nanocapsules) and C (control), the following groups were designated and treated with the suspensions: AMPH + FH; AMPH + H-NC; AMPH + B-NC; AMPH; and C. All groups received their first D,L-amphetamine administration (8 mg/kg ip) at time 0 (hour 0), except the control group (C), which received saline. After 30 min of AMPH or saline administration, the FH, H-NC and B-NC received a single injection of each nanocapsule suspension (0.2 mg/Kg body weight ip). The AMPH and C groups received a single injection of Tween 80® suspension (5% ip). Three, 6 and 9 h after the first AMPH administration, the rats received the second, third and fourth AMPH or saline administration. The duration of the experiment was 12 h, and every 3 h, a new dose of AMPH was administered. Two observers quantified the stereotyped head behavior every 15 min according to Ujike et al. [3] scale scores: 0, no head movement; 1, normal head movement and normal exploration; 2, increased rate of head movement with hyperactivity; 3, discontinuous repetitive and stereotyped up-down head movement; 4, continuous stereotyped head movement with occasional break; 5, continuous and intense stereotyped head movement at one location. Results are expressed as stereotyped behavior scores during the 3 h following each AMPH administration.

2.2.2. Experiment 2: motor side effects induced by acute haloperidol administration

Twenty-eight rats were divided in four groups ($n = 7$), as described below: (C group): received an ip injection of polysorbate 80 suspension 5% (v/v); (B-NC group): received an injection of blank nanocapsule suspension; (FH group): received an injection of free haloperidol suspension; (H-NC group): received an injection of haloperidol-loaded nanocapsules suspension. All drugs were administered at the dose of 0.2 mg/kg ip, and the behavioral tests were performed 1 h after the administration as follows:

(A) *Catalepsy*: Only the haloperidol-treated rats (FH and H-NC groups) were individually placed on a wire inclined grid (45° relative to the bench top) and observed for 60 s. The amount of time spent in this atypical position was recorded for three times, with an interval of 5 min between them. If the animal did not move, it was removed from the grid and returned to it. If it did not move within 60 s, it was removed again and returned to the grid. At the end, the mean time spent by the rat without moving was calculated for each test.

(B) *Oral dyskinesia (OD)*: Immediately after the catalepsy test, the rats were allocated in mirrored individual cages, allowing the assessment when the animal was facing away from the observer. OD was quantified by the frequency of vacuous chewing movement (VCM), which was recorded for three sets of 5 min with 5-min intervals. Observers were blind to the treatment.

2.2.3. Experiment 3: motor side effects induced by sub-chronic haloperidol administration

Twenty-eight rats were divided in the same four groups ($n = 7$) described above (C, B-NC, FH and H-NC). All drugs were administered in the dose of 0.2 mg/kg ip once a day for 28 days. The same behavioral tests (measurement of catalepsy and OD) were performed on the 7th, 14th, 21st and 28th days of treatment. On these days, the drugs were administrated after the behavioral tests, differently from Experiment 2.

2.3. Statistical analysis

AMPH-induced stereotyped behavior data (experiment 1) were analyzed by Kruskal–Wallis followed by Mann–Whitney *U*-test. Differences among the three observed times after each AMPH administration were analyzed by Wilcoxon matched pairs test. Acute and sub-chronic haloperidol-induced OD and catalepsy measurement data (experiments 2 and 3, respectively) were analyzed by one-way ANOVA followed by Duncan's multiple range test, if necessary. Differences among the groups at the same time were analyzed by paired samples *t*-test. A value of $p < 0.05$ was considered as statistically significant.

3. Results and discussion

The physicochemical characteristics of polymeric nanocapsules are shown in Table 1. All formulations appeared macroscopically homogeneous similar to a milky bluish opalescent fluid (Tyndall effect). Haloperidol was efficiently encapsulated (95 ± 1%) using the method of interfacial deposition of a preformed polymer (poly-ε-caprolactone), which was chosen due to its biodegradability and biocompatible properties. Both nanocapsule suspensions (B-NC and H-NC) presented particles in the sub-micrometric range (between 200 and 300 nm), low polydispersity (⩽0.25), negative zeta potentials, acid pH values and drug content near 100% of the theoretical (0.25 mg/mL). These values are in agreement with the diameters observed for nanocapsules prepared using the preformed polymers by the interfacial deposition method [4]. The negative zeta potential values (∼−8 mV) are a consequence of the particle coating with polysorbate 80, presenting a negative surface density of charge due to the presence of oxygen atoms in their molecules.

Efforts have been made by our group to reduce the motor disorders induced by haloperidol [5]. Studies have demonstrated that

Table 1
Physicochemical characteristics of blank and haloperidol-loaded nanocapsules (B-NC and H-NC).

Formulation	Particle size (nm)	PDI[a]	Zeta potential (mV)	pH	Drug content (mg/mL)
B-NC	280 ± 15	0.25 ± 0.1	−8.3 ± 0.5	6.3 ± 0.2	–
H-NC	230 ± 22	0.14 ± 0.1	−8.1 ± 0.2	6.5 ± 0.2	0.25 ± 0.2

Mean ± SD: represents the variation between the different batches ($n = 3$).
[a] PDI: polydispersity index.

drug-loaded nanocarriers are an efficient tool in drug delivery [6], promoting its permeation across the blood–brain barrier [7] and suggesting their use to deliver haloperidol to the brain. The results of experiment 1 show the head movement scores of AMPH-treated rats (Table 2). AMPH treatment increased the head movements at the three observed times after each AMPH administration, when compared to the control. Wilcoxon pairs test showed that the stereotyped behavior was modified by time after each AMPH administration. In addition, AMPH reached its maximum effect 1 h after each administration and this effect began to decrease 3 h later, indicating a new administration for psychosis maintenance. This procedure was thus performed every 3 h for 12 h for a full view of the responses to the haloperidol treatments (H-NC and FH) and proved to be adequate to evaluate the antipsychotic efficacy of haloperidol.

The effects of H-NC versus FH solution on the percentage of AMPH-induced stereotyped behavior are shown in Fig. 1. Kruskal–Wallis analysis revealed significant differences at the three observed times after the 1st ($p < 0.001$), 2nd ($p < 0.001$), 3rd ($p < 0.001$) and 4th ($p < 0.001$) AMPH administration, respectively. Rats treated with B-NC showed no reduction in head movements at any observation following each AMPH administration. On the other hand, both H-NC and FH showed reduced behavioral scores at all observed times after the 1st and 2nd AMPH administration. Following the 3rd dose, only the H-NC group showed decreased movement scores. From 2 h after this AMPH administration, only H-NC reduced the movements in relation to the B-NC group. Regarding the differences between the two haloperidol-treated groups, the H-NC group showed a greater decrease in stereotyped movements than the FH group in almost all observations, except 3 h after the 1st and 1 h after the 4th AMPH administration. The Wilcoxon test indicated that the effects of B-NC, FH and H-NC on the AMPH-induced stereotyped behavior were modified by time after each AMPH administration (Fig. 1A–C). After the last dose of AMPH, the stereotyped behavior was reduced at 3 h only, for all groups (B-NC, FH and H-NC) (Fig. 1D).

Table 2
Stereotyped behavior amphetamine (AMPH) induced in rats ($n = 7$). Data are represented in scores scale.

AMPH administrations	Hours	Head movement	
		C group	A group
	1	0.2 (0/0.5)	3.5 (3/3.5)
1	2	0.2 (0/0.2)	5 (4/5)[a]
	3	NS	3.7 (3.7/4)
	1	0 (0/0.2)	5 (5/5)
2	2	NS	4.2 (4/5)
	3	NS	3.2 (3/3.5)[a,b]
	1	NS	4.7 (4.5/4.7)
3	2	NS	5 (4.7/5)
	3	NS	3.7 (3.5/4)[a,b]
	1	NS	5 (5/5)
4	2	NS	4.5 (4.5/4.5)
	3	NS	3.7 (3.5/4)

NS: No stereotypy. Data are expressed as median (lower/upper quartile). The lowercase letters show significant differences among the times within the same treatment.
[a] Different from 1 h.
[b] Different from 2 h.

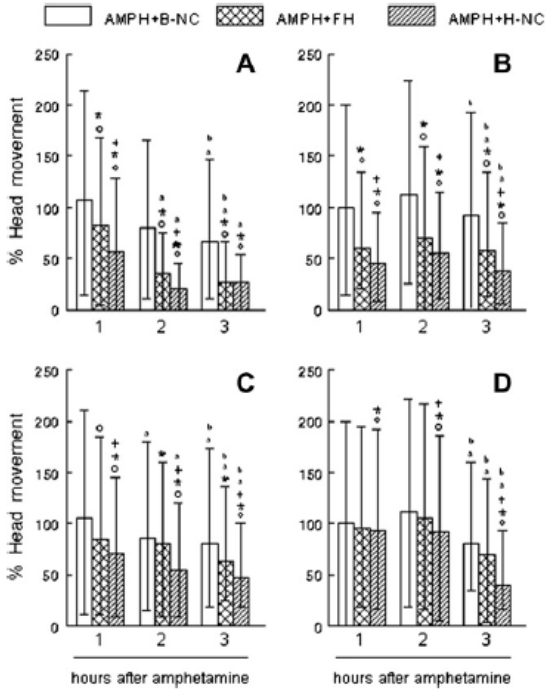

Fig. 1. Effect of blank nanocapsules (B-NC), free haloperidol (FH) and haloperidol-loaded nanocapsules (H-NC) on amphetamine (AMPH)-induced stereotyped behavior in rats ($n = 7$). The behavioral evaluation was expressed as stereotyped behavior scores for 3 h following the first (A), second (B), third (C) and fourth (D) AMPH administration. Data are expressed as median (lower/upper quartile) of % amphetamine-induced stereotyped behavior. The lowercase letters show significant differences among the times within the same AMPH administration; symbols show significant difference among treatments within the same AMPH administration. *Different from AMPH; different from AMPH+B-NC group; +different from AMPH+FH group; [a]different from 1 h; [b]different from 2 h.

With a single sub-therapeutic dose (0.2 mg/kg), haloperidol-loaded nanocapsules showed higher antipsychotic effects evidenced by the decrease in AMPH-induced stereotyped movements when compared to the FH group. As haloperidol is a potent antipsychotic, a low dose was chosen to allow the observation of the efficacy of H-NC compared to FH at the same dose. In our study, both groups treated with haloperidol (H-NC and FH) decreased the AMPH-induced stereotyped behavior, but the H-NC group showed a more prolonged antipsychotic action at the equivalent dose. These findings are in agreement with a recent study [8], which also showed stronger biological effects in brain using drug delivery systems composed of polymeric nanocapsules. In order to evaluate if this prolonged antipsychotic effect was accompanied

by adverse effects, motor effects were monitored after acute and sub-chronic haloperidol courses (experiments 2 and 3, respectively). The motor side effects induced by acute haloperidol treatment (H-NC or FH) are shown in Fig. 2. Duncan's test of the OD data showed an increase of 95% in the VCM frequency ($p < 0.05$) after the administration of FH when compared to the C group (C). H-NC or B-NC showed unchanged behavior in relation to the C group regarding this orofacial parameter (Fig. 2A). Duncan's test showed that the H-NC treatment led to a decrease in the immobility time (31%) in relation to the FH group ($p < 0.05$) (Fig. 2B). Taken together, these results showed that FH treatment caused extrapyramidal effects, such as OD and catalepsy. On the other hand, the H-NC group showed less motor side effects than the group treated with the free drug. Subsequently, we evaluated the motor side effects induced by sub-chronic haloperidol treatment (H-NC and FH) (Fig. 3). Post hoc tests of OD showed a significant increase in the VCM frequency after FH administration when compared to the C group ($p < 0.05$). The groups treated with H-NC and B-NC showed unchanged behavior in relation to the C group regarding this orofacial parameter at all analyzed times (Fig. 3A). Paired t-test indicated that there were no significant modifications in the C, B-NC and H-NC groups along the time. However, there was a significant increase in the orofacial parameter in the FH group at day 21 when compared to days 7 and 28.

Duncan's test of catalepsy showed a lower immobility time (23%) in the H-NC-treated group than in the FH group at day 28 ($p < 0.05$) (Fig. 3B). The paired test indicated that both FH and H-NC groups showed an increase in catalepsy time at days 21 and 28 when compared to day 7 ($p < 0.05$) (Fig. 3B). These results were similar to those observed after H-NC acute administration and confirm that H-NC causes less extrapyramidal effects than FH does. The mechanism behind drug delivery into the brain by colloidal carriers remains uncertain. Studies have given support to the role of polysorbates on the endocytosis processes. Polysorbate acts mainly as an anchor for the apolipoprotein-overcoated nanoparticles and thus would mimic lipoprotein particles and could interact with and then be taken up by the brain capillary endothelial cells via receptor-mediated endocytosis and/or transcytosis [9]. In fact, nanoparticles coated with polysorbate adsorb apoliprotein E. Apart

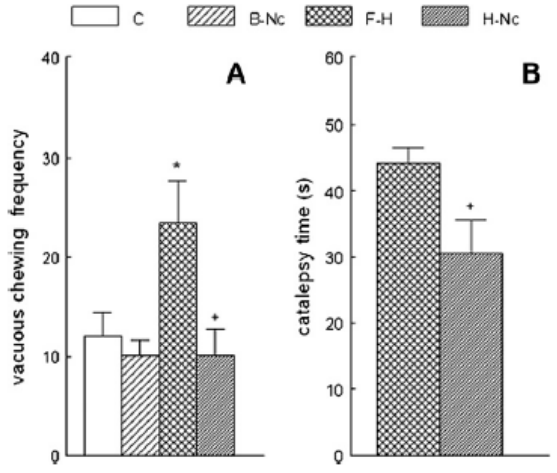

Fig. 2. Acute effect of free haloperidol or haloperidol-loaded nanocapsules on the development of vacuous chewing movements (A) and catalepsy (B) in rats. Both behavioral parameters were evaluated 1 h after haloperidol administration. Data are expressed as mean + SEM ($n = 7$). C – control group; B-NC – blank nanocapsules group; FH – free haloperidol; H-NC – haloperidol-loaded nanocapsules. *Different from C group; *different from FH group.

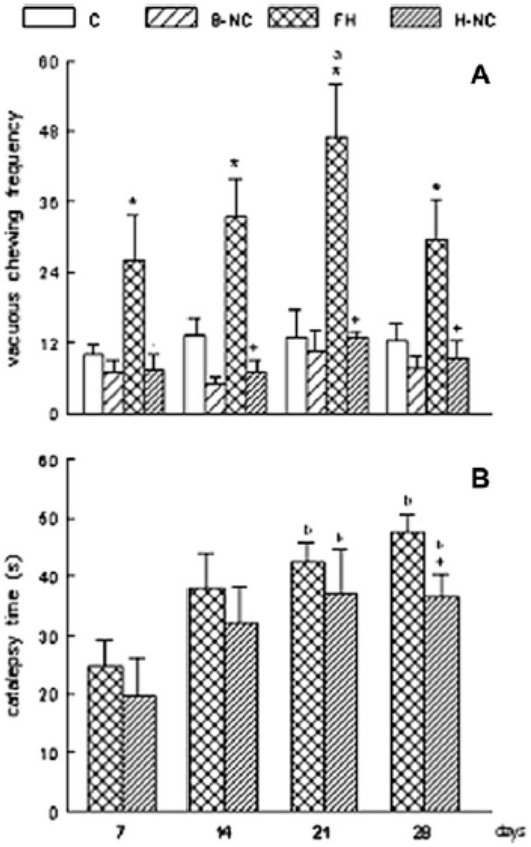

Fig. 3. Sub-chronic effect of free haloperidol or haloperidol-loaded nanocapsules on the development of vacuous chewing movements (A) and catalepsy (B) in rats. Both behavioral parameters were evaluated on days 7, 14, 21 and 28 after daily haloperidol administration. Data are expressed as mean + SEM ($n = 7$). C – control group; B-NC – blank nanocapsules group; FH – free haloperidol; H-NC – haloperidol-loaded nanocapsules. The lowercase letters show significant differences among the times within the same treatment; symbols show significant difference among treatments within the same time. *Different from C group; *different from FH group; [a]different from days 7 and 28; [b]different from day 7.

from this, the surfactant polysorbate 80 is an inhibitor of P-glycoprotein, representing an important constituent of the BBB. The molecular basis for the barrier function of the BBB is a group of drug efflux transporters such as P-glycoprotein, which hinder the access of some drugs into the CNS. The mechanism involved in this barrier function is by extruding drugs from the brain and is a major obstacle for many pharmacological agents. Furthermore, the coating of polymeric nanoparticles by polysorbate 80 can change their particle surface (hydrophobic to hydrophilic), avoiding their opsonization by plasma proteins. Thus, H-NC could maintain haloperidol blood levels for a longer time, explaining its effect for at least 12 h, i.e., about three additional hours when compared to the free suspension.

So, the most important result was the significant decrease in the motor side effects after H-NC administrations, which are commonly related to dopamine receptors blockade at the nigro-striatal system. Thereby, our results suggest that H-NC targeted the drug to the mesocorticolimbic region (related to psychotic symptoms), reaching the nigro-striatal system in lower concentration and minimizing the extrapyramidal disorders. Haloperidol levels in brain

dopaminergic regions need to be studied in order to confirm this hypothesis.

The feasibility of delivering drugs into the brain using polymeric nanoparticles may open new perspectives for the treatment of diseases such as schizophrenia, mainly by the possibility of achieving their biological activity at low doses. Recently, the prolonged antipsychotic effects and reduced extrapyramidal effects of risperidone-loaded nanoparticles, an atypical antipsychotic, were demonstrated [10]. Here, we are demonstrating for the first time the design of a nanomedicine as an alternative to the administration of haloperidol, which is the most widely used drug to treat mental disorders, and its nanoencapsulation in polymeric systems is a promising therapeutic tool.

In summary, our data clearly demonstrated the feasibility of preparing H-NC to improve its therapeutic efficacy. The antipsychotic effect of H-NC was maintained for a longer time, and the acute and sub-chronic extrapyramidal motor disorders were reduced when compared to the administration of FH suspension. The exact mechanisms related to these findings should be investigated in further studies.

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References

[1] A. Rieux, V. Fievez, M. Garinot, Y.J. Schneider, V. Préat, Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach, J. Control. Release 116 (2006) 1–27.
[2] H. Fessi, F. Puisieux, J.-P. Devissaguet, N. Ammoury, S. Benita, Nanocapsule formation by interfacial polymer deposition following solvent displacement, Int. J. Pharm. 55 (1989) r1–r4.
[3] H. Ujike, K. Akiyama, S. Otsuki, D2 but not D1 dopamine agonists produce augmented behavioral response in rats after subchronic treatment with methamphetamine or cocaine, Psychopharmacology 102 (1990) 459–464.
[4] S.R. Schaffazick, A.R. Pohlmann, T. Dalla-Costa, S.S. Guterres, Freeze-drying polymeric colloidal suspensions: nanocapsules, nanospheres and nanodispersion. A comparative study, Eur. J. Pharm. Biopharm. 56 (2003) 501–505.
[5] R.C.S. Barcelos, D.M. Benvegnú, N. Boufleur, P. Reckziegel, L. Muller, C. Pase, T. Emanuelli, M.E. Burger, Effects of ω-3 essential fatty acids (ω-3 EFAs) on motor disorders and memory dysfunction typical neuroleptic-induced: behavioral and biochemical parameter, Neurotox. Res. 17 (2010) 228–237.
[6] M. Teixeira, M.J. Alonso, M.M.M. Pinto, C.M. Barbosa, Development and characterization of PLGA nanospheres and nanocapsules containing xanthone and 3-methoxyxanthone, Eur. J. Pharm. Biopharm. 59 (2005) 491–500.
[7] S.R. Schaffazick, I.R. Siqueira, A.S. Badejo, D.S. Jornada, A.R. Pohlmann, C.A. Netto, S.S. Guterres, Incorporation in polymeric nanocapsules improves the antioxidant effect of melatonin against lipid peroxidation in mice brain and liver, Eur. J. Pharm. Biopharm. 69 (2008) 64–71.
[8] B. Wilson, M.K. Samanta, K. Santhi, K.P.S. Kumar, N. Paramakrishnan, B. Suresh, Poly(n-butylcyanoacrylate) nanoparticles coated with polysorbate 80 for the targeted delivery of rivastigmine into the brain to treat Alzheimer's disease, Brain Res. 1200 (2008) 159–168.
[9] J. Kreuter, D. Shamenkov, V. Petrov, P. Ramge, K. Cychutek, C. Koch-Brandt, R. Alyautdin, Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood–brain barrier, J. Drug Target 10 (2002) 317–325.
[10] M.S. Muthu, M.K. Rawat, A. Mishra, S. Singh, PLGA nanoparticle formulations of risperidone: preparation and neuropharmacological evaluation, Nanomedicine 5 (2009) 323–333.

3.2 Manuscrito 1

HALOPERIDOL-LOADED POLYSORBATE-COATED POLYMERIC NANOCAPSULES DECREASE ITS ADVERSE MOTOR SIDE EFFECTS AND OXIDATIVE STRESS MARKERS IN RATS

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Haloperidol-loaded polysorbate-coated polymeric nanocapsules decrease its adverse motor side effects and oxidative stress markers in rats

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Running head: **Haloperidol nanocapsules: pharmacological study**

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ABSTRACT

Haloperidol is the most widely used antipsychotic drug in the treatment of psychiatric disorders. Despite its satisfactory therapeutic effect, its chronic use is related to severe motor side effects. Here, we investigate the incidence of motor side effects of haloperidol-loaded nanocapsules when compared to free haloperidol and the relation with oxidative stress (OS) development. Both vehicle (B-NcFO) and haloperidol loaded polysorbate-coated nanocapsules suspension (H-NcFO) prepared with fish oil as core showed uniform and rounded particles, nanometric size, negative zeta potential, low polydispersity indices and high encapsulation efficiency. Wistar rats received a single dose of free haloperidol (FH), B-NcFO or H-NcFO (0.2mg/kg ip) and were submitted to acute motor side effects evaluation 1h after the injection. Lower catalepsy time and oral dyskinesia were observed in H-NcFO-treated group than in FH group; however, both formulations decreased animals' locomotor activity. In a experiment performed subchronically, rats injected daily with H-NcFO (0.2mg/kg-ip) for 28 days showed decreased oral dyskinesia frequency and catalepsy time and no impairment on locomotor activity as compared to FH group (0.2mg/kg-ip). FH group showed higher OS, as observed by increased lipid peroxidation and reduced glutathione levels and catalase activity in extrapyramidal region. Our findings showed that nanocapsules may be an efficient form to prevent or minimize haloperidol motor side effects, which are related to OS development, ameliorating psychiatric patients' quality of life.

Keywords: Haloperidol, polymeric nanocapsule, extrapyramidal effects, orofacial dyskinesia, oxidative stress.

1. Introduction

Haloperidol is a typical antipsychotic drug used in the treatment of schizophrenia, mania and neurological disorders and recognized for its potency, specificity and long action (Mutschler et al., 1995). Its mechanism of action is mediated by blockade of D2 dopamine receptors in brain (Creese et al. 1976).

The antipsychotic efficacy of haloperidol is somewhat compromised by the tendency to cause acute and chronic extrapyramidal movement disorders such as parkinsonism, akathisia, dystonias, and tardive dyskinesia, which is characterized by repetitive, often irreversible and disabling involuntary movements (Andreassen and Jorgensen, 2000). Haloperidol and its metabolites also produce abnormal movements in animals, e.g. oral dyskinesia (OD), which has been associated with morphological alterations and oxidative stress (OS) in extrapyramidal brain regions (Andreassen and Jorgensen, 2000; Bürger et al., 2006; Fachinetto et al., 2007; Naidu et al., 2003). In this sense, the pathophysiology of movement disorders induced by haloperidol has been related to oxidative damage (Bürger et al., 2006; 2005a) and neurotoxicity, which are closely related to increase of turnover rate of dopamine (by blockade of presynaptic receptors), leading to production of reactive substances as a by-product of its metabolism (Andreassen and Jorgensen, 2000). Furthermore, the brain is more susceptible to oxidative damage when compared to other organs or systems (Halliwel and Gutteridge 1999), mainly because it contains high levels of membrane lipids, excitotoxic amino acids, low levels of antioxidant defenses, and autoxidizable neurotransmitters. Experimentally, several studies conducted in our laboratory have shown a relationship between haloperidol-induced movement disorders and OS, as well as beneficial effects of antioxidant compounds (Barcelos et al., 2010; Bürger et al., 2006; 2005a; Colpo et al., 2007; Trevizol et al, 2011).

Although more recent atypical neuroleptics do not cause extrapyramidal motor disorders, their clinical use remains restricted to a minority of patients because of their high cost, body weight gain, and development of diabetes and blood dyscrasias requiring intensive monitoring (Meltzer et al., 1995). For these reasons, typical neuroleptics such as haloperidol are still the most widely used antipsychotic drugs in treatment of schizophrenia and other psychiatric disorders (Ponto et al., 2010).

Polymeric nanoparticles have attracted considerable attention as potential drug delivery systems, increasing therapeutic efficacy and reducing side effects of a variety of drugs associated with these systems (Beck et al., 2005; 2006; Bernardi et al., 2009; Mora-Huertas et al., 2010). Polymeric nanoparticles present a size ranging from 10 to 1000 nm and

are employed to carry drugs by incorporation or absorption (Garcia-Garcia et al, 2005). Carried by nanoparticles, drugs can be released at controlled rates and to specific body sites to obtain accurate delivery, which will enhance their therapeutic efficacy and reduce their toxicity and side effects (Kreuter et al., 2002; Mohanraj and Chen, 2006).

Polysorbate-coated nanoparticles were previously reported as efficient carriers capable of transporting the loaded drugs across blood-brain barrier (BBB) after administration, being interesting drug delivery systems to the brain (Bernardi et al., 2010; 2009; Xin-Hua et al., 2011; Wang et al., 2009). Furthermore, polymeric nanoparticles can be used as parenteral controlled release systems to prolong the availability of drugs that freely penetrate the BBB but have a short duration of action in the central nervous system (Friese et al., 2000).

It was demonstrated that the association of antipsychotic risperidone with nanoparticles prolonged its therapeutic effect and reduced catalepsy time in mice (Muthu and Singh, 2008; Muthu et al., 2009). Recently we reported better therapeutic efficacy and longer time of action by haloperidol nanocapsules in a pseudo-psychosis animal model (Benvegnú et al., 2011). Moreover, this formulation also caused minor acute and subchronic extrapyramidal side effects, as observed by reduction of OD and shorter time of catalepsy in rats. In addition, since extrapyramidal motor side-effects are related to haloperidol treatment, in the present study we aimed to observe the acute and subchronic motor side effects induced by free and nanoencapsulated haloperidol and their relationship to OS markers in dopaminergic brain regions involved in the development of movement disorders. For this, we developed haloperidol-loaded polysorbate-coated nanocapsules containing fish oil (FO) rich in n-3 fatty acids, whose antiapoptotic actions (Bazan, 2007) have shown beneficial effects in animal models of movement disorders (Barcelos et al., 2011; 2010) We hypothesized that this haloperidol nanoformulation may be an alternative to overcome some drawbacks associated with the therapeutic use of free haloperidol.

2. Materials and methods

2.1. Chemicals

Haloperidol was obtained from Galena (Campinas-SP, Brazil). Fish oil capsules (Achè-Guarulhos-SP, Brazil), containing 1g oil/capsule with 120mg of DHA and 180mg of EPA were used. Poly(ε-caprolactone) and sorbitan monostearate (Span 60®) were obtained from Sigma (Tatuapé-SP, Brazil). Polysorbate 80 was acquired from Delaware (Porto Alegre, RS, Brazil). All other chemicals and solvents used were of pharmaceutical or HPLC grade and used as received.

2.2. Animals

Wistar adult male rats (200±50g) were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (22–23°C) and on a 12h-light/dark cycle with lights on at 7:00 am. The experimental protocol of this study was approved by Animal Ethical Committee of Universidade Federal de Santa Maria (CIETEA- 22/2010), which is affiliated to CONCEA, and was adhered to the "Principles of Laboratory Animal Care'' (NIH publication) and international rules of ethics in research.

2.3. Preparation of haloperidol-loaded polysorbate-coated nanocapsules suspensions containing fish oil

Nanocapsules (H-NcFO) were prepared by interfacial deposition of preformed polymer, as described by Fessi et al. (1989). Briefly, the organic solution consisted of FO (0.8 mL), poly(ε-caprolactone) (250 mg), haloperidol (6.3 mg), sorbitan monostearate (192 mg) and acetone (67 mL), which were poured under magnetic stirring into an aqueous solution (134 mL) containing polysorbate 80 (192 mg). Acetone was removed and the suspension concentrated by evaporation under reduced pressure. The final volume of the suspension was adjusted to 25 mL (0.25 mg/mL of haloperidol). Blank nanocapsules (B-NcFO) were prepared using the same protocol described above, but omitting the presence of the drug. Haloperidol free suspension (0.25 mg/mL) was prepared in an aqueous solution using 5% (w/v) of polysorbate 80.

2.4. Physicochemical characterization of nanocapsules

Particle sizes and polydispersity indices (n=3) were measured by photon correlation spectroscopy after adequate dilution (1:500 v/v/) of an aliquot of the suspension in distilled water (Zetasizer Nanoseries ZEN 3600, Malvern Instruments, UK). *Zeta potential* was determined using the same equipment, but after dilution of the samples in 10 mmol L^{-1} NaCl aqueous solution. Drug content (mg/mL) was determined (n=3) after dissolution of nanocapsules in acetonitrile (1 mL of suspension to 5 mL of acetonitrile) and assayed by high performance liquid chromatography (HPLC), according to previously described and validated protocol (Benvegnú et al., 2011), using a Shimadzu instrument (LC-10AVP Pump, UV-VIS SPD-10AVP Module, Class-VP Software, Shimadzu, Tokyo, Japan). Encapsulation efficiency was determined by the ultrafiltration-centrifugation technique. So, the concentration of free drug was determined in the clear supernatant after this technique

(Ultrafree-MC® 10,000 MW, Millipore, Bedford, USA). Encapsulation efficiency (%) was calculated by the difference between total and free drug concentrations determined in the nanocapsule suspension (drug content) and in the ultrafiltrate, respectively, using the HPLC method described above. pH values of suspensions were determined by immersion of the electrode directly in the suspension using a calibrated potentiometer (MPA-210 Model, MS-Tecnopon, São Paulo, Brazil), at room temperature. In addition, the morphological analysis was conducted by transmission electron microscopy (TEM; Jeol, JEM 1200 Exll, Centro de Microscopia Eletrônica, UFRGS) operating at 80 kV. For this analysis, the diluted suspension was deposited in Formvar-Carbon support film on specimen grid (Electron Microscopy Sciences) and negatively stained with 2% (w/v) uranyl acetate solution.

2.5. In vivo experiments

2.5.1. Experiment 1: Motor side effects induced by acute haloperidol administration

Twenty-eight rats were randomly divided in four groups (n=7), and treated with single injection (ip) of aqueous solution containing polysorbate 80 5% (v/v) (negative control, group-C), haloperidol aqueous dispersion (free haloperidol, group-FH), blank nanocapsules (blank nanocapsules, group-B-NcFO) and haloperidol-loaded nanocapsules (haloperidol nanocapsules, group-H-NcFO). All suspensions were administered in the dose of 0.2 mg/Kg-ip and the behavioral tests were performed one hour after the administration, according to the methodology described below:

2.5.1.1. Oral dyskinesia

Animals were individually placed in cages (20x20x19 cm^3) containing one mirror under the floor and one behind the back wall of the cage to allow behavioral quantification when the animal was facing away from the observer. To quantify the occurrence of oral dyskinesia, the incidence of vacuous chewing movement frequency (VCM) was recorded for three sets of 5 minutes with 5-minute intervals. Observers were blind to the treatment. In a preliminary study (using five control and ten haloperidol-treated rats) of interrater reliability, we found that the use of this method of observation and definition for the parameters evaluated usually results in 94% agreement between three different observers.

2.5.1.2. Locomotor activity

In order to evaluate the reduction of haloperidol-induced motor activity, rats' spontaneous locomotor activity was quantified just after the first interval of oral dyskinesia.

Animals were placed individually in the center of an open-field arena (40 x 40 x 30 cm) with black plywood walls and a white floor divided into nine equal squares, as described by Kerr et al. (2005). The number of lines crossing and the number of rearings was recorded for 5 min. The crossing and rearing numbers were indicators of locomotor and exploratory activity, respectively.

2.5.1.3. Catalepsy time

This behavioral test was adapted from Rocha et al. (1997). Catalepsy was measured using a wire grid (25x9x30 cm^2) inclined 45^o relative to the bench top. This behavioral parameter is characterized by positional passivity, which is observed through failure to correct an uncomfortable imposed posture. Each rat was placed with its forepaws near the edge of the grid and the amount of time spent in this atypical position was recorded for three times, with an interval of 5 min between them. All the rats treated with haloperidol (group FH and group H-NcFO) were individually placed on the inclined grid and observed for 60 seconds. At the end of the three replications, the mean time spent by the rat without moving was calculated for each test.

2.5.2. Experiment 2: Motor side effects induced by subchronic haloperidol administration and oxidative stress markers

Twenty-eight rats were randomly divided in the same four groups (n =7) described above (C, B-NcFO, FH and H-NcFO). All drugs were administered (ip) in the dose of 0.2 mg/Kg/mL once a day, for 28 consecutive days. The same behavioral tests (oral dyskinesia, catalepsy time and locomotor activity) described above were performed 7, 14, 21, and 28 days after treatment initiation, immediately before administration of suspensions.

At day 29 animals were euthanized by cervical decapitation after anesthesia with sodium pentobarbital (50 mg/kg body weight-ip). Brains were removed and cut coronally at the caudal border of the olfactory tubercle. Cortex, hippocampus and striatum were removed; Posterior areas containing the right and left substantia nigra were carefully dissected according to the coordinates of the Atlas (Paxinos and Watson, 2007): Anterior-posterior (AP) –4 to -6 mm; dorsal-ventral (DV) 7 to 8.8 mm; lateral (L) 2 to 3 mm, using external landmarks as delimiters of the region. Dissected nigral area, cortex, hippocampus and striatum were homogenized in 10 v (w/v) of 0.1 M Tris-HCl, pH 7.4, centrifuged at 1300 g (10 min) and the supernatants were used for OS biomarkers determination.

2.5.2.1. Lipid Peroxidation measure

Lipid peroxidation (LP) was estimated by quantification of thiobarbituric acid reactive species (TBARS). LP was measured through the pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde (MDA) at 100ºC, measured spectrophotometrically at 535 nm, according to Ohkawa et al. (1979). Results were expressed as ηmol MDA/g tissue.

2.5.2.2. Antioxidant defenses

Reduced gluthatione (GSH) tissue content was determined after reaction with 5,5'-dithiobis-(2-nitrobenzoic acid). The yellow color formed was read at 412 nm, according to Boyne and Ellman (1972). A standard curve using cysteine was used to calculate the content of GSH in tissue samples, expressed as μmol GSH/g tissue.

Catalase (CAT) activity was spectrophotometrically quantified in tissues by the method of Aebi (1984), which involves monitoring the disappearance of H_2O_2 in the presence of cell homogenate (pH 7 at 25°C) at 240 nm. The enzymatic activity was expressed in μmol H_2O_2/min/g tissue.

2.6. Statistical Analysis

Data were analyzed by two way ANOVA [(4 (FH/ BNcFO/ HNcFO/ C) x 4 periods of behavioral quantifications)] followed by Duncan's multiple range test, when required. Pearson's correlation coefficient was calculated between behavioral parameters (VCM, catalepsy, crossing and rearing) and substantia nigra LP (TBARS levels). Data were analyzed using Statistica (11.0 version) and expressed as mean±S.E.M. Significance was considered when $p<0.05$.

3. Results

3.1. Physicochemical characteristics of polymeric nanocapsules are shown in Table 1 and Figure 1.

All formulations appeared macroscopically homogeneous and their aspects were similar to a milky bluish opalescent fluid (Tyndall effect). Both nanocapsule suspensions (B-NcFO and H-NcFO) presented particles in the submicrometric range (between 200 and 300 nm), low polydispersity (≤ 0.25), negative zeta potentials and neutral pH values (Table 1). The nanometric size population represents more than 97% of the particle size distribution for

both formulations. Furthermore, H-Nc showed drug content of 0.249 mg/mL and excellent encapsulation efficiency (95.09±0.43%). TEM analysis showed uniform and rounded particles (Figure 1).

3.2. Motor side effects induced by haloperidol acute treatment (haloperidol-loaded nanocapsules or free haloperidol) are shown in Figure 2.

Oral dyskinesia: VCM frequency was increased by 310% after free haloperidol (FH) administration ($p<0.05$) in relation to control (C group). Both nanocapsule suspensions (H-NcFO and B-NcFO groups) showed orofacial movements unchanged, which were similar to control (Fig. 2A).

Catalepsy measurement: In this behavioral evaluation, C and B-NcFO groups showed no catalepsy. Therefore, they were not included in the results and discussion sections. Duncan post hoc tests showed that H-NcFO treatment led to a decrease in immobility time (10%) in relation to FH group ($p<0.05$) (Fig. 2B).

Locomotor activity: Haloperidol-treated rats (FH and H-NcFO groups) demonstrated lower locomotor activity than C group. In fact, FH and H-NcFO groups presented a decrease of 86% and 84% in crossing and 90% and 84% in rearing, respectively (Fig. 2C and 2D).

3.3 Motor side effects induced by subchronic haloperidol treatment (haloperidol-loaded nanocapsules and free haloperidol) are shown in Figures 3 and 4.

Oral dyskinesia: Post hoc test showed a significant increase in VCM frequency in FH-treated group in relation to control ($p<0.05$), while both nanocapsule suspensions (H-NcFO and B-NcFO) did not affect this parameter in animals at all analyzed times (Fig 3A). Paired sample T-test indicated that vehicle- (C), blank nanocapsules- (B-NcFO) and free haloperidol- (FH) treated rats showed no changes in VCM frequency over time, but between days 7 and 14, haloperidol-loaded nanocapsules (H-NcFO) treatment increased this orofacial parameter.

Catalepsy time measurement: H-NcFO-treated rats showed lower immobility time than FH-treated onesat all analyzed times ($p<0.05$) (Fig. 3B). Paired sample T-test indicates that H-NcFO group increased catalepsy time at day 28 as compared to day 7.

Locomotor activity: FH-treated rats showed decreased crossing and rearing behavior at days 28 and 21, respectively, in relation to control. Both nanocapsule suspension (B-NcFO and H-NcFO) treatments did not affect locomotor activity in relation to control (Fig. 4A and 4B). Paired comparison indicates that FH group showed a significant decrease in crossing at

days 21 and 28as compared to day 7and in rearing at days 21 and 28as compared to days 7 and 14.

3.4. Oxidative stress and antioxidant defenses parameters of rats treated with subchronic haloperidol treatment (haloperidol-loaded nanocapsules and free haloperidol) are shown in Table 2.

Duncan post hoc test revealed that FH group showed an increase in TBARS levels in striatum and substantia nigra, a decrease in GSH levels in striatum and a reduction in CAT activity in substantia nigra as compared to C group. H-NcFO group showed a decrease in TBARS levels in striatum and substancia nigra as well as an increase in GSH levels in striatum in relation to FH group.

3.5. Correlations of rats treated with subchronic haloperidol (haloperidol-loaded nanocapsules containing fish oil and free haloperidol) are shown in Table 3.

Pearson's correlation coefficient analyses revealed a significant positive correlation of substantia nigra TBARS levels with VCM frequency and catalepsy duration. In addition, there was a negative correlation of substantia nigra TBARS levels with locomotor parameters (crossing and rearing), confirming that haloperidol-induced motor disturbances are closely related to OS, verified through LP.

4. Discussion

Our previous study showed that antipsychotic effects of haloperidol-loaded nanocapsules was maintained for longer time and more efficient and the motor side effects were reduced in relation to free haloperidol (Benvegnú et al., 2011), but up to now no study had evaluated the oxidative status in extrapyramidal brain regions of rats treated with this formulation.

Many studies have demonstrated that drug-loaded nanocarriers are an efficient tool in drug delivery, enhancing therapeutic effects and reducing adverse side effects (Beck et al., 2005; 2006; Bernardi et al., 2009; Fontana et al., 2011; Wu et al., 2008). Furthermore, these systems are able to promote permeation of drugs across the BBB, as previously demonstrated by several authors (Bernardi et al., 2009; 2010; Xin-Hua et al., 2011; Wang et al., 2009), suggesting their use as an alternative to haloperidol delivery to the brain.

Here, haloperidol was efficiently encapsulated in polymeric nanocapsules using FO as oily core. The physicochemical properties of the new formulation are similar to the formulation previously developed using medium chain triglycerides as oil core (Benvegnú et al., 2011). So, FO did not have any interference in the system, showing adequate nanotechnological characteristics (size, zeta potential, encapsulation efficiency and morphology) as well as drug delivery systems (pH and drug content). Although the zeta potential can be considered low (in module), it is important to highlight that these polymeric suspensions are stabilized by the presence of the polysorbate layer at the particle/water interface, acting as a steric stabilizer (Jager et al., 2007).

Despite the advances observed in recent years, the mechanism involved in the transport of drug-loaded polymeric nanocapsule into the brain remains uncertain (Garcia-Garcia et al., 2005). Polysorbate 80 could play a special role as anchor between nanoparticles and the apolipoprotein, especially ApoE. Nanoparticles combined with the apolipoprotein are considered as LDL, and LDL receptor-mediated transcytosis brings drug-loaded nanoparticles across the BBB (Kreuter et al., 2002). In addition, polysorbate 80 could also inhibit the efflux system, especially P-glycoprotein (Kreuter et al., 2005), which hinder the access of some drugs into the central nervous system. However, the exact mechanism involved in this transport remains unclear.

Motor side effects were monitored after acute and subchronic haloperidol treatment. Free haloperidol-treated group showed extrapyramidal side effects, evidenced by increase of OD frequency and catalepsy time, as well by locomotor impairment, reinforcing previous reports of our group (Barcelos et al., 2010; Colpo et al., 2007; Trevizol et al., 2011) and other research groups (Naidu et al., 2003; Zhang et al., 2007).

Excitotoxicity and OS have been closely related to development of haloperidol-induced extrapyramidal side effects (Tsai et al., 1998). In line with this, haloperidol administration can increase dopamine turnover, which reacts with molecular oxygen to form dopamine quinones depleting glutathione and generating reactive species during this process (Andreassen and Jorgensen, 2000). In addition, blockade of striatal dopamine receptors can increase extracellular glutamate (Bürger et al., 2005b), which in turn contributes to OS development (Tsai et al., 1998). Then, the involvement of excitotoxicity and particularly OS in haloperidol-induced motor disorders has been intensely studied by our group (Barcelos et al., 2010; Bürger et al., 2005a, b; Colpo et al., 2007; Teixeira et al., 2011) seeking to better understand the pathophysiology of extrapyramidal disturbances induced by typical antipsychotics such as haloperidol, in order to minimize and/or prevent these symptoms. Of

particular importance, findings of the present study demonstrated that free haloperidol administration caused a significant LP increase in striatum and substantia nigra, which are brain regions closely involved in movement disorders. Concomitantly, antioxidant defenses such as GSH levels and CAT activity were also reduced by free haloperidol treatment in striatum and substantia nigra, respectively. Contributing to our findings, a positive correlation between movement disorders and TBARS levels in substantia nigra were observed as well. Taken together, these findings confirm OS involvement in development of extrapyramidal disorders such as haloperidol-induced OD. On the other hand, haloperidol loaded-nanocapsules-caused fewer motor side effects than free haloperidol did, with no significant increase of brain LP or changes in the antioxidant defense system.

In fact, recently we showed that haloperidol nanocapsules were able to increase and prolong its antipsychotic effect with a reduction of motor side effects (Benvegnú et al., 2011). Then, different preparations of slow release such as haloperidol decanoate and osmotic minipumps are currently available in the antipsychotic therapeutic arsenal, which achieve controlled release and stable plasma levels, but unfortunately, they still are able to induce long-term motor side effects (Andreassen & Jorgensen, 2000). In this sense, searches for new formulations that may minimize or delay the serious motor side effects haloperidol-induced are emerging. Here, we are showing for the first time that the beneficial effects of nanoencapsulated haloperidol on the motor system may result from lower oxidative damages in extrapyramidal brain regions, when compared to free drug. In fact, these brain structures themselves may be key contributors to OS development, mainly because they are rich in dopamine that generates reactive metabolites by autoxidation and deamination (by monoamino oxidase-MAO) (See, 1991). Considering that the efficacy of haloperidol-loaded nanocapsules showed a more pronounced activity in a animal model of pseudo-psychosis (Benvegnú et al., 2011) and that drug-loaded polysorbate 80 coated-nanocapsules reaches more efficiently the brain compared to the administration of the non-encapsulated drug (Bernardi et al., 2009; Frozza et al., 2010), the hypothesis of the lower delivery of haloperidol to the brain can be refuted. This way, we can suggest two hypothesis: i) an alteration in the distribution of haloperidol in the different brain areas to explain the decrease in the extrapyramidal motor disorders after the administration of its nanoencapsulated form. This modified biodistribution could contribute to lower generation of reactive species and OS, evidenced here by minor LP in these dopaminergic regions; ii) haloperidol-loaded nanocapsules allows a sustained and continued delivery of drug, without causing its excessive bioaccumulation in brain extrapyramidal area, differently from FH, whose brain delivery

occurs instantaneously and may generate a burst effect, facilitating drug accumulation in the same area of the basal ganglia, and evoking motor side effects, as shown in the present study. In fact, according to different authors, polymeric nanoparticles promote targeted drug delivery (Nagarwal et al., 2009; Zhu et al., 2009), higher selectivity and lower systemic side effects (Beck et al., 2005; 2006; Gao et al., 2010) in relation to free drug. However, more studies involving both pharmacokinetic parameters such as biodistribution and tecidual levels of haloperidol in brain dopaminergic areas are needed to confirm on or both hypothesis proposed here.

5. Conclusion

Our study showed that haloperidol loaded-nanocapsules were able to minimize motor side effects as well as oxidative damages in extrapyramidal brain regions. To our knowledge, this is the first pharmacological study demonstrating beneficial effects of haloperidol nanoencapsulation on the oxidative status and related to behavioral evaluations. We believe that this new polymeric nanocapsule system can be considered in psychiatry in order to reduce OS-related movement disorders, ameliorating the quality of life of patients who need haloperidol. Further studies are necessary to investigate the haloperidol levels in different brain regions as a function of time.

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References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 5, 121–126.

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

Barcelos, R.C.S., Benvegnú, D.M., Boufleur, N., Reckziegel, P., Muller, L., Pase, C., Emanuelli, T., Burger, M.E., 2010. Effects of omega-3 essential fatty acids (omega-3 EFAs) on motor disorders and memory dysfunction typical neuroleptic-induced: behavioral and biochemical parameter. Neurotox. Res. 17, 228-237.

Barcelos, R.C.S., Benvegnú, D.M., Boufleur, N., Pase, C.S., Teixeira, A.M., Reckziegel, P., Emanuelli, T., Rocha, J.B.T., 2011. Burger ME. Short term dietary fish oil supplementation improves motor deficiencies related to reserpine-induced parkinsonism in rats. Lipids 46, 143-149.

Bazan, N.G., 2007. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. Clin Nutr. Metab. Care 10, 136–141.

Beck, R.C.R., Pohlmann, A.R., Benvenutti, E.V., Dalla Costa, T.C., Guterres, S.S., 2005. Nanostructure-coated diclofenac-loaded microparticles: Preparation, morphological characterization, in vitro release and in vivo gastrointestinal tolerance. J. Braz. Chem. Soc. 16, 1233-1240.

Beck, R.C.R., Haas, S.E., Guterres, S.S., Ré, M.I, Benvenutti, E.V , Pohlmann, A.R., 2006. Nanoparticle-coated organic-inorganic microparticles: experimental design and gastrointestinal tolerance evaluation. Quím. Nova 29, 990-996.

Benvegnú, D.M., Barcelos, R.C.S., Boufleur, N., Reckziegel, P., Pase, C.S., Ourique, A.F., Beck, R.C.R., Burger, M.E., 2011. Haloperidol-loaded polysorbate-coated polymeric nanocapsules increase its efficacy in the antipsychotic treatment in rats. Eur. J. Pharm. Biopharm. 77, 332-336.

Bernardi, A., Braganhol, E., Jaeger, E., Figueiro, F., Edelweiss, M.I., Pohlmann, A.R., Guterres, S.S., Battastini, A.M.O., 2009. Indomethacin-loaded nanocapsules treatment reduces in vivo glioblastoma growth in a rat glioma model. Cancer Lett. 281, 53-63.

Bernardi, A., Frozza, R.L, Horn, A.P., Campos, M.M., Calixto, J.B., Salbego, C., Pohlmann, A.R., Guterres, S.S.; Battastini, A.M.O., 2010. Protective effects of indomethacin-loaded

nanocapsules against oxygen-glucose deprivation in organotypic hippocampal slice cultures: Involvement of neuroinflammation. Neurochem. Int. 57, 629–636.

Boyne, A.F., Ellman, G.L., 1972. A methodology for analysis of tissue sulfhydryl components. Anal Biochem. 46, 639-653.

Burger, M.E., Rocha, J.B.T., Fachinetto, R., Callegari, L., 2005a. Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain. Pharmacol. Biochem. Behav. 81, 608-615.

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

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

Colpo, G., Trevizol, F., Teixeira, A.M., Pereira, R.P., Rocha, J.B.T., Burger, M.E., 2007. Ilex paraguariensis has antioxidant potential and attenuates haloperidol-induced orofacial dyskinesia and memory dysfunction in rats. Neurotox. Res. 12, 1-10.

Creese, I., Burt, D.R., Snyder, S.H., 1996. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. J. Neuropsychiatry Clin. Neurosci. 8, 223-226.

Fachinetto, R., Villarinho, J.G., Wagner, C., Pereira, R.P., Burger, M.E., Rocha, J.B.T., Ferreira, J., Calixto, J.B., 2007. Valeriana officinalis does not alter the orofacial dyskinesia induced by haloperidol in rats: Role of dopamine transporter. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 1478-1486.

Fessi, H., Puisieux, F., Devissaguet, J.P., Ammoury, N., Benita, S., 1989. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int. J. Pharm. 55, r1–r4.

Fontana, M.C., Rezer, J.F.P., Coradini, K., Leal, D.B.R., Beck, R.C.R., 2011. Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. Eur. J. Pharm. Biopharm. 79, 241-249.

Friese, A., Seiller, E., Quack, G., Lorenz, B., Kreuter, J., 2000. Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system. Eur. J. Pharm. Biopharm. 49, 103–109.

Frozza, R. L. Bernardi, A., Paese, K.; Hoppe, J. B. ; Silva, T.; Battastini, A. M.; Pohlmann, A. R.., Guterres, S. S.; Salbego, C. 2010. Characterization of trans-resveratrol-loaded lipid-core nanocapsules and tissue distribution studies in rats. Journal of Biomedical Nanotechnology. 6, 694-703.

Gao, Y., Xu, P., Chen, L., Li, Y., 2010. Prostaglandin E_1 encapsulated into lipid nanoparticles improves its anti-inflammatory effect with low side-effect. Int. J. Pharm. 387, 263-271.

Garcia-Garcia, E., Andrieux, K., Gil, S., Couvreur, P., 2005. Colloidal carriers and blood–brain barrier (BBB) translocation: a way to deliver drugs to the brain? Int. J. Pharm. 298, 274–292.

Halliwel, B., Gutteridge, J.M.C., 1999. Free radicals in biology and medicine. Oxford, University Press.

Jager, A., Stefani, V., Guterres, S.S., Pohlmann, A.R., 2007. Physico-chemical characterization of nanocapsule polymeric wall using fluorescent benzazole probes. Int. J. Pharm. 338, 297–305.

Kerr, D.S., Bevilaqua, L.R., Bonini, J.S., Rossato, J.I., Kohler, C.A., Medina, J.H., Izquierdo, I., Cammarota, M., 2005. Angiotensin II blocks memory consolidation through an AT(2) receptor dependent mechanism. Psychopharmacology 179, 529-535.

Kreuter, J., Shamenkov, D., Petrov, V., Ramge, P., Cychutek, K., Koch-Brandt, C., Alyautdin, R., 2002. Apolipoprotein-mediated transport of nanoparticle-bound drugs across the blood–brain barrier. J. Drug Target. 10, 317–325.

Kreuter, J., 2005. Application of nanoparticles for the delivery of drugs to the brain. Int. Congr. Ser. 1277, 85–94.

Meltzer, H.Y., Thompson, P.A., Lee, M.A., Ranjan, R., 1995. Neuropsychologic déficits in schizophrenia: relation to social function and effect of antipsychotic drug treatment. Neuropsychopharmacology 14, 27S-33S.

Mohanraj, V., Chen, Y., 2006. Nanoparticles – a review. Trop. J. Pharm. Res. 5, 561-573.

Mora-Huertas, C.E., Fessi, H., Elaissari, A., 2010. Polymer-based nanocapsules for drug delivery. Int. J. Pharm. 385, 113-142.

Mutschler, E., Derendorf, H., Schafer-Korting, M., Elrod, K., Estes, K.S., 1995. Drug actions: basic principles and therapeutic aspects. Medpharm Scientific Publishers, Stuttgart, pp. 116–125.

Muthu, M.S., Singh, S., 2008. Studies on biodegradable polymeric nanoparticles of risperidone: in vitro and in vivo evaluation. Nanomedicine 3, 305-319.

Muthu, M.S., Rawat, M.K., Mishra, A., Singh, S., 2009. PLGA nanoparticle formulations of risperidone: preparation and neuropharmacological evaluation. Nanomedicine 5, 323-333.

Naidu, P.S., Singh, A., Kulkami, S.K., 2003. Quercetin, a bioflavonoid attenuates haloperidol-induced orofacial dyskinesia. Neuropharmacology 44, 1100-1106.

Nagarwal, R.C., Kant, S., Singh, P.N., Maiti P., Pandit J.K., 2009. Polymeric nanoparticulate system: A potential approach for ocular drug delivery. J. Control. Release 136, 2-13.

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

Ourique, A. F., Azoubel, S., Ferreira, C.V., Silva, C.B., Marchiori, M.C.L., Pohlmann, A.R., Guterres, S.S., Beck, R.C.R., 2010. Lipid-core nanocapsules as a nanomedicine for parenteral administration of tretinoin: development and in \vitro antitumor activity on human myeloid leukaemia cells. J. Biomed. Nanotechnol. 6, 214-223.

Paxinos, G.; Watson, C., 2007. The rat brain in stereotaxic coordinates, sixth ed. Elsevier, Amsterdam.

Ponto, T., Ismail, N.I., Abdul Majeed, A.B., Marmaya, N.H., Zakaria, Z.A., 2000. A prospective study on the pattern of medication use for schizophrenia in the outpatient pharmacy department, Hospital Tengku Ampuan Rahimah, Selangor, Malaysia. Methods Find. Exp. Clin. Pharmacol. 32, 427-32.

Rocha, J.B.T., Santos, J.E.T., Rocha, L.K., Kleinpaul, E.R., 1997. Undernutrition during suckling changes the sensitivity to haloperidol and chlorpromazine in two behavioural measures in weaning rats. Pharmacol. Toxicol. 80, 114–123.

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

Teixeira, A.M., Müller, L.G., Reckziegel, P., Boufleur, N., Pase, C.S., Villarinho, J.G., Fachinetto, R., Ferreira, J., Rocha, J.B.T., 2011. Bürger ME. Beneficial effects of an innovative exercise model on motor and oxidative disorders induced by haloperidol in rats. Neuropharmacology 60, 432-438.

Trevizol, F., Benvegnú, D.M., Barcelos, R.C.S., Pase, C.S., Segat, H., Dias, V.T., Dolci, G.S., Boufleur, N., Reckziegel, P., Bürger, M.E., 2011. Comparative study between two animal models of extrapyramidal movement disorders: prevention and reversion by pecan nut shell aqueous extract. Behav. Brain Res. 221, 13-18.

Tsai, G., Goff, D.C., Change, R.W., Flood, J., Bair, L., Coyle, J.T., 1998. Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. Am. J. Psychiatry 155, 1207– 1213.

Wang, C.X., Huang, L.S., Hou, L.B., Jiang, L., Yan, Z.T., Wang, Y.L., Che, Z.L., 2009. Antitumor effects of polysorbate-80 coated gemcitabine polybutylcyanoacrylate nanoparticles in vitro and its pharmacodynamics in vivo on C6 glioma cells of a brain tumor model. Brain Res. 1261, 91-99.

Wu, T.W., Yen, F.L., Lin, L.T., Tsai, T.R., Lin, C.C., Cham, T.M., 2008. Preparation, physicochemical characterization, and antioxidant effects of quercetin nanoparticles. Int. J. Pharm. 346, 160–168.

Xin-Hua, T., Xiao-Ning, L., Feng, W., Huang, Z., Wang, P., Ren, L., Diao, Y., 2011. Enhanced brain targeting of temozolomide in polysorbate-80 coated polybutylcyanoacrylate nanoparticles. Int. J. Nanomedicine 6, 445-452.

Zhang, Y., Xu, H., He, J., Yan, B., Jiang, W., Li, X,. Li, X.M., 2007. Quetiapine reverses altered locomotor activity and tyrosine hydroxylase immunoreactivity in rat caudate putamen following long-term haloperidol treatment. Neurosci. Lett., 420, 66-71.

Zhu, L., Ma, J., Jia, N., Zhao, Y., Shen, H., 2009. Chitosan-coated magnetic nanoparticles as carriers of 5-Fluorouracil: Preparation, characterization and cytotoxicity studies. Colloids Surf. B: Biointerfaces 68, 1-6.

Legends for Figures

Figure 1. Transmission electron microscopy (TEM) images of haloperidol- nanocapsule. A-image on the left: bar =1200 nm (200.000x); B - image on the right: bar = 50 nm (400.000x).

Figure 2. Acute effect of free or nanoencapsulated haloperidol (0.2 mg/kg, ip). on vacuous chewing movements (VCM) frequency (A), catalepsy time (B), crossing (C) and rearing (D) numbers in rats (n=7). These observations were performed 1 h after a single haloperidol administration. C-control; B-NcFO-blank nanocapsules; FH-free haloperidol; H-NcFO-haloperidol-loaded nanocapsules. Data are expressed as mean±S.E.M (n=7). *Indicates significant difference from C group; $^{+}$Indicates significant difference from FH group (P<0.05 for all comparisons).

Figure 3. Subchronic effects of daily administration of free or nanoencapsulated haloperidol (0.2 mg/Kg/mL-ip once a day, for 28 days) on vacuous chewing movement (VCM) frequency (A) and catalepsy time (B) in rats (n=7). These observations were performed at 7, 14, 21 and 28 days after the first injection. C-control; B-NcFO-blank nanocapsules; FH-free haloperidol; H-NcFO-haloperidol-loaded nanocapsules. Data are expressed as mean±S.E.M. (n=7). Symbols indicate significant difference between treatments at same observed time: *difference from C group; $^{+}$difference from FH group. Letters indicate significant difference between observed times in the same treatment: ^{a}difference from 7 day *(*P<0.05 for all comparisons).

Figure. 4. Subchronic effects of daily administration of free or nanoencapsulated haloperidol (0.2 mg/Kg/mL-ip once a day, for 28 days) on crossing (A) and rearing behavior (B) in rats (n=7). These observations were performed at 7, 14, 21 and 28 days after the first injection. C-control; B-NcFO-blank nanocapsules; FH-free haloperidol; H-NcFO-haloperidol-loaded nanocapsules. Data are expressed as mean±S.E.M. (n=7). Symbols indicate significant difference between treatments at same observed time: *difference from C group; $^{+}$difference from FH group. Letters indicate significant difference between observed times in the same treatment: ^{a}difference from day 7; ^{b}difference from day 14 (P<0.05 for all comparisons).

Table 1. Physicochemical characteristics of blank and haloperidol loaded-nanocapsules containing fish oil (B-NcFO and H-NcFO).

Nanocapsules	Drug Content (mg/mL)	Particle size (nm)	PDI[a]	Zeta Potential (mv)	pH
B-NcFO	-	290.14±14.4	0.23±0.02	-12.43±1.6	7.05±0.2
H-NcFO	0.25±0.002	261.40±3.52	0.21±0.00	-13.23±0.7	7.46±0.1

Mean±S.D.: represents the variation between the different batches (n=3).
[a] PDI: polydispersity index.

Figure 1:

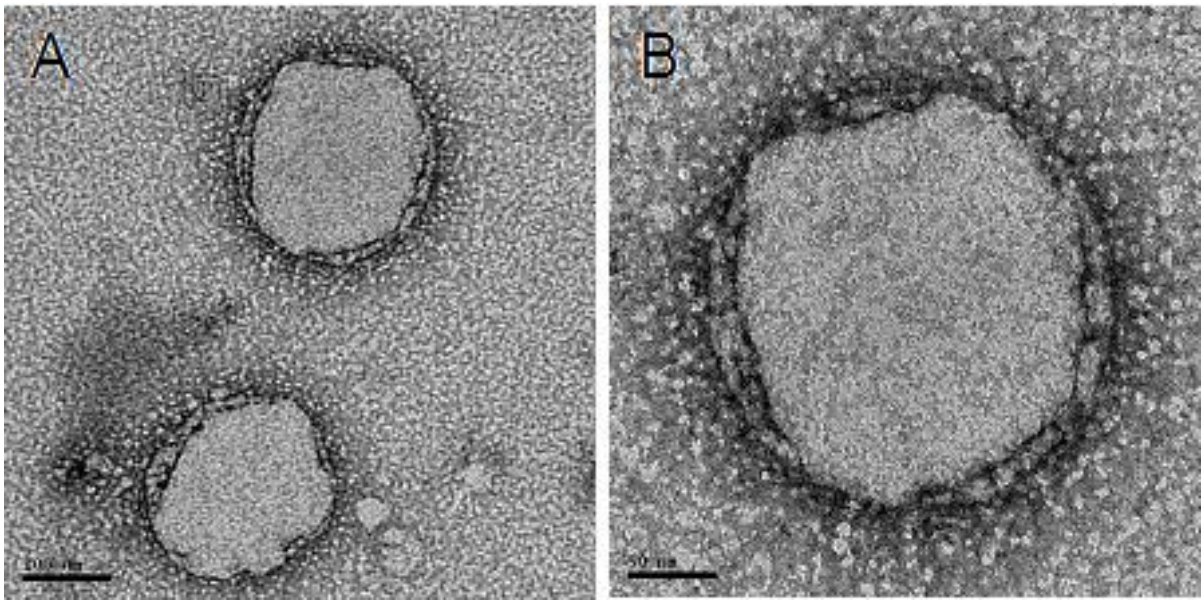

Figure 2:

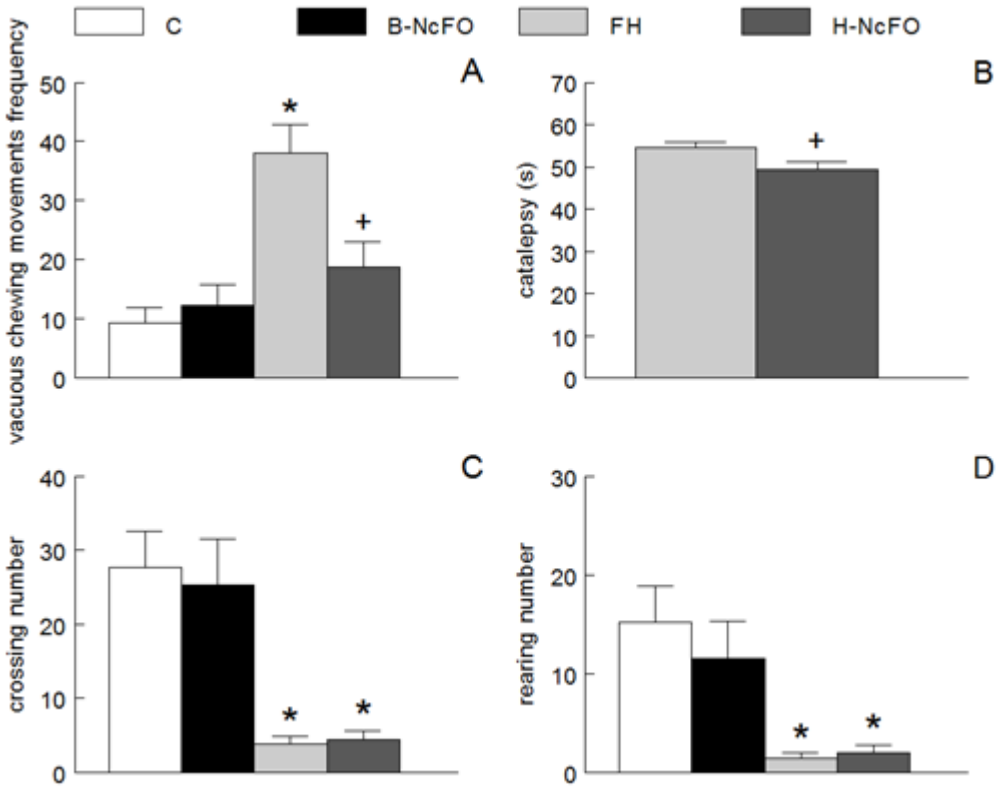
C
B-NcFO
FH
H-NcFO
A
B
C
D
vacuous chewing movements frequency
catalepsy (s)
crossing number
rearing number
0
10
20
30
40
50
60
70
*
+

Figure 3:

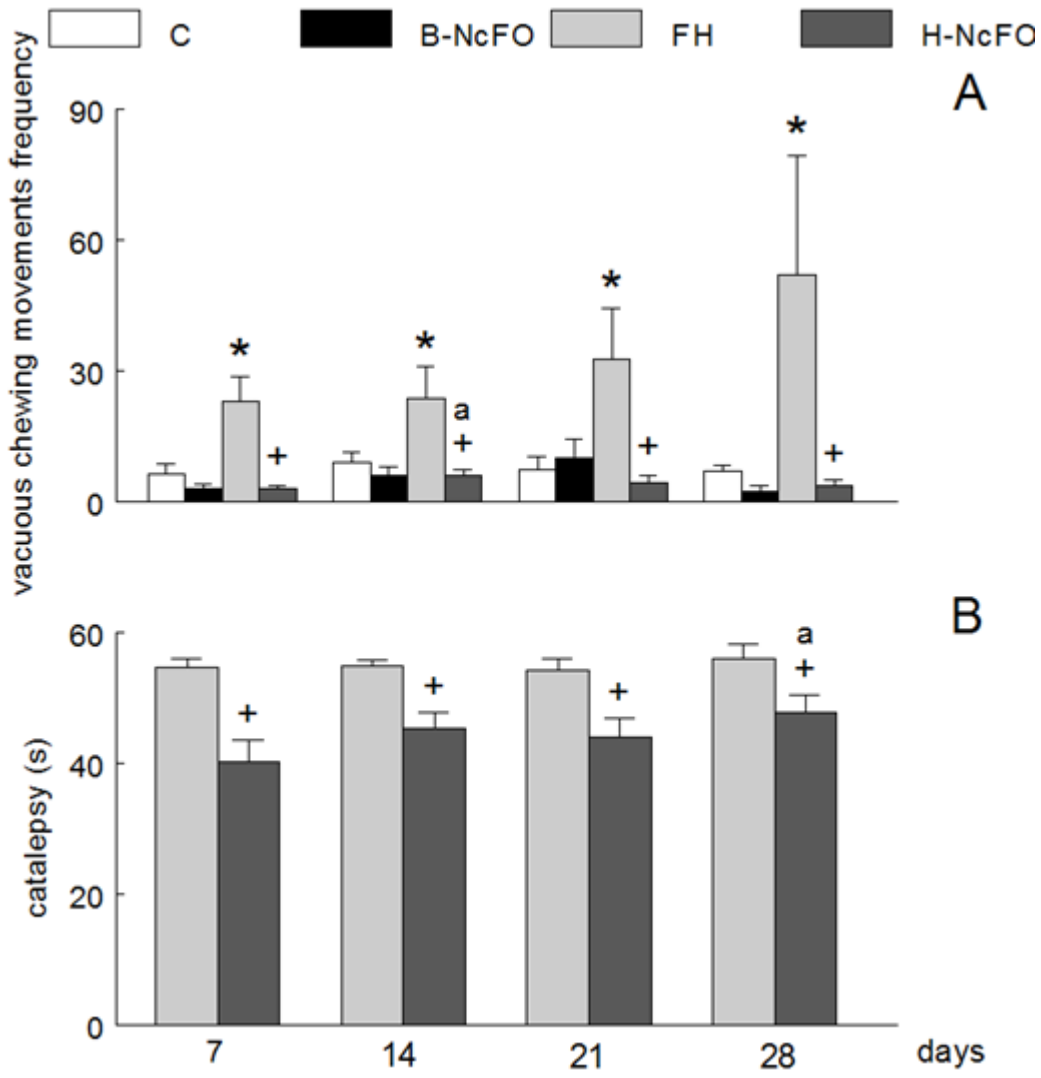
C
B-NcFO
FH
H-NcFO
A
vacuous chewing movements frequency
90
60
30
0
*
*
*
*
a
+
+
+
+
B
catalepsy (s)
60
40
20
0
+
+
+
a
+
7
14
21
28
days

Figure 4:

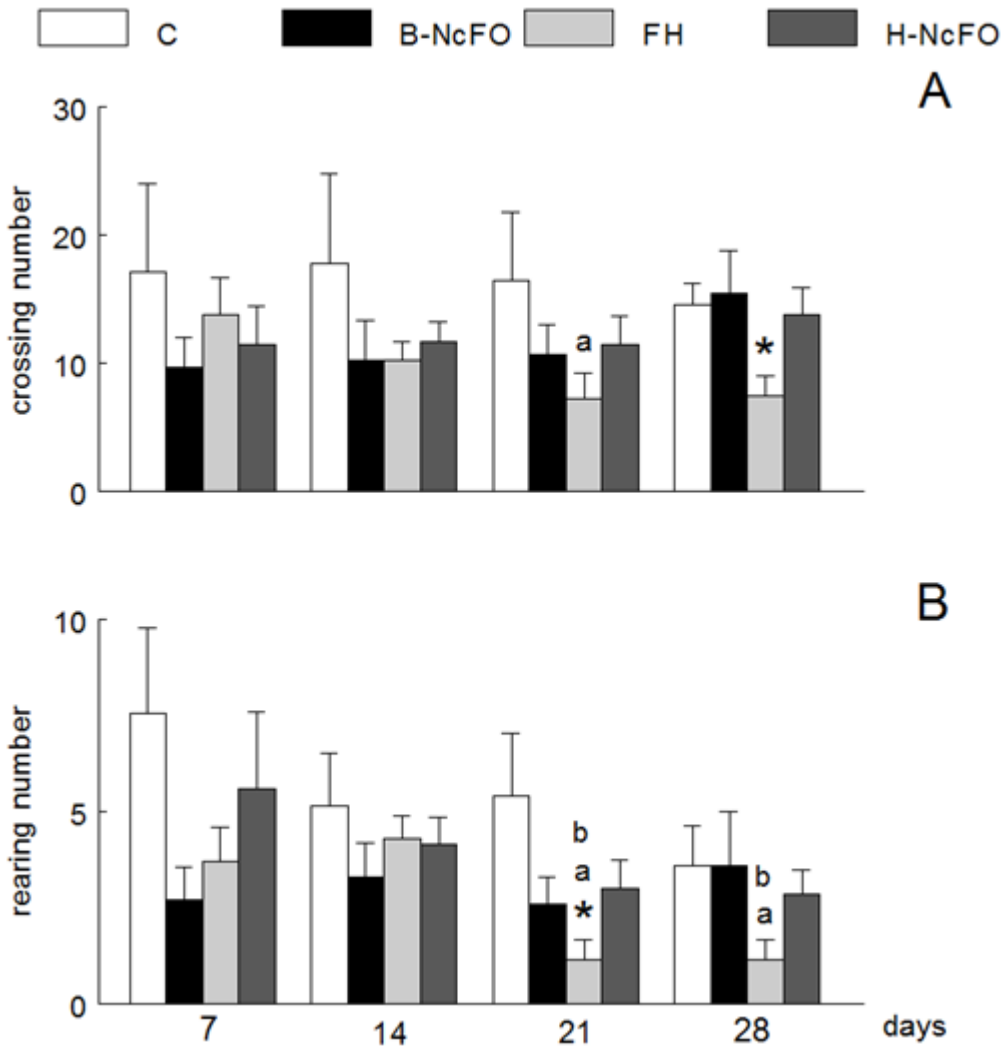
C
B-NcFO
FH
H-NcFO
A
crossing number
30
20
10
0
a
*
B
rearing number
10
5
0
b
a
*
b
a
7
14
21
28
days

Table 2. Biochemical measures from lipid peroxidation measured by thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and catalase (CAT) of rats.

Parameter	Tissue	C	B-NcFO	FH	H-NcFO
TBARS	Cortex	164.91±10.61	167.02±20.20	198.62±7.74	161.67±17.32
	Striatum	126.96±09.88	118.37±23.07	177.15±23.97*	97.41±12.33^{+}
	Substantia Nigra	107.84±18.42	180.33±39.81	342.80±17.51*	179.06±37.86^{+}
GSH	Cortex	1.88±0.17	2.16±0.11	1.76±0.29	1.98±0.11
	Striatum	1.97±0.11	1.69±0.22	1.12±0.20*	1.83±0.26^{+}
	Substantia Nigra	1.66±0.36	1.43±0.43	0.94±0.07	1.17±0.24
CAT	Cortex	224.44±46.68	163.89±16.05	165.34±2.49	206.42±31.50
	Striatum	262.80±41.98	202.85±26.72	210.89±24.69	225.91±25.24
	Substantia Nigra	342.60±26.50	233.66±26.55	208.19±18.31*	307.72±63.20

Values are expressed as mean±S.E.M. (n=7).

C-control; B-Nc-blank nanocapsules; FH-free haloperidol; H-Nc-haloperidol-loaded nanocapsules.

Units-TBARS: ηmol MDA/g tissue; GSH: μmol GSH/g tissue; CAT: μmol H_2O_2/min/g tissue.

*Indicates significant difference from C group; +Indicates significant difference from FH group (P<0.05 for all comparisons).

Table 3. Linear regression analysis between substantia nigra TBARS levels and behavioural parameters in rats treated with free haloperidol or haloperidol-loaded nanocapsules. Linear regression was evidenced by Pearson's correlation coefficients (n=7).

Behavioural parameter	*R*	*p*
Substantia nigra TBARS-VCM	0.599	0.007
Substantia nigra TBARS-Catalepsy	0.708	0.022
Substantia nigra TBARS-Crossing	-0.579	0.009
Substantia nigra TBARS-Rearing	-0.511	0.025

Linear regression was evidenced by Pearson's correlation coefficients (n=7).

3.3 Manuscrito 2

EFFECTS OF FISH AND GRAPE SEED OILS AS CORE OF HALOPERIDOL-LOADED NANOCAPSULES ON ORAL DYSKINESIA IN RATS: BEHAVIORAL AND BIOCHEMICAL EVALUATIONS

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Status: **pronto para submissão**

Effects of fish and grape seed oils as core of haloperidol-loaded nanocapsules on oral dyskinesia in rats: Behavioral and biochemical evaluations

Running head: **Haloperidol nanocapsules: pharmacological study**

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ABSTRACT

Background and purpose: Haloperidol is a widely used antipsychotic, despite the severe motor side effects resulting from its chronic use. This study was carried out to compare oral dyskinesia (OD) induced by different formulations of haloperidol-loaded nanocapsules containing caprylic/capric triglycerides (H-NcCCO), fish oil (H-NcFO) or grape seed oil (H-NcGSO) as core, as well as free haloperidol (FH).

Experimental approach: Haloperidol-loaded lipid-core nanocapsules formulations were prepared and physicochemical characterized. After, the formulations (0.5 mg/kg-ip) were administered to rats for 28 days. OD was evaluated acutely and subchronically. Cell viability and free radical generation in cortex and substantia nigra were evaluated at the end of behavioral observations.

Key results: All formulations presented satisfactory physicochemical parameters, however the H-NcGSO formulation showed lower encapsulation efficiency. Acutely, all evaluated formulations were able to prevent development of OD in comparison to FH, except H-NcGSO, whose preventive effect was only partial. After subchronic treatment, all formulations of haloperidol-loaded nanocapsules prevented OD development in relation to free drug. In addition, H-NcFO and H-NcGSO were more effective than H-NcCCO and FH in cell viability preservation and control of free radical generation.

Conclusions and implications: Our findings showed that H-NcFO may be considered as the best formulation of haloperidol-loaded lipid-core nanocapsules, being able to prevent motor side effects associated with chronic use of antipsychotic drugs, as haloperidol.

Keywords: haloperidol, polymeric lipid-core nanocapsule, fish oil, grape seed oil, oral dyskinesia

Abbreviations: BBB-brain blood barrier, B-Nc-blank nanocapsules, CCO-caprylic/capric triglycerides, CNS-central nervous system, DA-dopamine, FA-fatty acids, FH-free haloperidol, FO-fish oil, GSO-grape seed oil, H-Nc-haloperidol loaded nanocapsules, OD-oral dyskinesia, OS-oxidative stress, PUFA-polyunsaturated fatty acids, SFA-saturated fatty acids.

1. Introduction

Haloperidol is a butyrophenone commonly used to treat schizophrenia. It is widely prescribed worldwide due to its high potency and low cost (Ponto et al., 2010). However, the use of this antipsychotic is limited by its strong tendency to produce extrapyramidal movement disorders such as Parkinsonism, akathisia, dystonia, and tardive dyskinesia (Creese et al., 1976). Haloperidol has been experimentally used to study movement disorders, observed by the generation of orofacial dyskinesia (OD) and catalepsy in animals (Andressen and Jorgensen, 2000; Barcelos et al., 2010; Trevizol et al., 2011), which occur due to the increased turnover rate of dopamine (DA) (by blockade of the pre-synaptic receptors) in the nigro-striatal brain area. The overproduction of reactive substances as a byproduct of DA metabolism is closely related to the development of oxidative stress (OS) and neurotoxicity (Andressen and Jorgensen, 2000; Zhu et al., 2004; Bürger et al., 2005a). Moreover, neuronal apoptosis was demonstrated *in vitro* (Ukai et al., 2004) and *in vivo* (Mitchell et al., 2002) after haloperidol administration.

In the last years, efforts have been made by our research group to understand and ameliorate the perspectives of the use of neuroleptic drugs. Several studies have shown the beneficial effects of natural antioxidants, synthesized compounds (Burger et al, 2005b; Burger et al., 2006; Fachinetto et al., 2007; Colpo et al., 2007; Trevizol et al., 2011; Busanello et al., 2011) and dietary n-3 fatty acids (FA) (Barcelos et al. 2010; 2011) by preventing or minimizing these adverse motor effects. Recently, as an alternative to avoid this serious problem, we reported greater therapeutic efficacy and longer time of action of haloperidol-loaded polysorbate-coated lipid-core nanocapsules, with minor acute and subchronic extrapyramidal side effects as observed by the prevention of OD and decreased time of catalepsy in rats (Benvegnú et al., 2011).

Novel drug delivery systems such as polymeric nanoparticles have attracted considerable attention by their innovative property of enhancing therapeutic efficacy (Ourique et al., 2011; Fontana et al. 2011; Bernardi et al., 2009; Ianiski et al., 2012) while minimizing side effects (Beck et al., 2005; Yen et al., 2008; Gao et al., 2010). These beneficial properties are due to these nanosystems' ability to release drugs at a controlled rate and in specific body sites (Nagarwal et al., 2009; Zhu et al., 2009). Of particular importance, drugs that present low brain penetration have shown facilitated transport across the brain blood barrier (BBB) when loaded by nanoparticles (Schaffazick et al., 2008; Bernardi et al., 2009; Frozza et al., 2010; Wohlfart et al., 2011). Furthermore, nanometric systems are able to prolong the action time of drugs that freely penetrate the brain but have a short action duration in the central

nervous system (CNS) (Friese et al., 2000), a property that has been shown in neuroleptic drugs (Muthu et al., 2009, Parikh et al., 2010, Seju et al., 2011).

The type of oily phase used as the core in preparation of polymeric nanocapsules can influence the mean particle size and polydispersity index due to differences in its viscosity, hydrophobic characteristic, and interfacial tension (Schaffazick et al., 2003; Bouchemal et al., 2004). Alternative vegetable oils have been assayed to prepare nanocapsules with satisfactory results (Dhanikula et al., 2007; Almeida et al., 2009; 2010, Flores et al., 2011). In addition, different oils with therapeutic activity can be used (Almeida et al., 2009, 2010), thus contributing to the pharmacological effects of the drug.

A recent study by Almeida et al., (2010) showed antioxidant property of rutin nanocapsules containing grape seed oil (GSO) *in vitro*. GSO contains chemical components rich in phenolic compounds, linoleic acid and tocopherols that present antioxidant activity (Baydar et al., 2007). However, the therapeutic properties of this oil in the CNS are poorly known and therefore deserve more research. In contrast, fish oil (FO) is already recognized for this contribution in CNS disorders (Wainwright, 2002). Recently, our research group showed the beneficial effect of FO supplementation on haloperidol- and reserpine-induced movement disorders in rats (Barcelos et al., 2010; Barcelos et al., 2011), and we also demonstrated a reduction in extrapyramidal side effects closely related with OS biomarkers in rats treated with haloperidol-loaded nanocapsules containing FO (Benvegnú et al., 2012). FO is rich in n-3 polyunsaturated fatty acids (PUFA) (Stansby et al, 1969), which contribute to the maintenance of the histological, anatomical and biochemical integrity of neuronal membranes phospholipids (Zararsiz et al., 2006) due to attenuation of apoptotic processes associated with OS (Bazan 2006, 2007).

Given that haloperidol-loaded lipid-core nanocapsules have shown fewer motor side effects in rats (Benvegnú et al., 2011), the aim of this study was to compare the physicochemical properties of haloperidol-loaded nanocapsules containing different oils, and the capacity of these formulations to induce extrapyramidal disturbances, together with a biochemical analysis of the brain areas involved in movement disorders.

2. Materials and methods

2.1. Chemicals

Haloperidol was obtained from Galena (Campinas-SP, Brazil). FO capsules containing 1g oil/capsule, with 120mg of DHA and 180mg of EPA, were acquired from Achè®-Guarulhos-SP, Brazil. Poly(ε-caprolactone) and sorbitan monostearate (Span 60®) were

obtained from Sigma (Tatuapé-SP, Brazil). Caprylic/capric triglyceride (CCO) mixture was supplied by Brasquim (Porto Alegre-RS, Brazil). Polysorbate 80 (Tween 80®) and GSO were acquired from Delaware (Porto Alegre, RS, Brazil). All other chemicals and solvents used were of pharmaceutical or HPLC grade and used as received.

2.2. Animals

Adult male Wistar rats (360±35g) were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (22–23°C) and on a 12h-light/dark cycle with lights on at 7:00 a.m. The experimental protocol of this study was approved by the Animal Ethical Committee of the Universidade Federal de Santa Maria (CIETEA- 22/2010), which is affiliated to CONCEA in accordance with international rules of ethics in research.

2.3. Poly(ε-caprolactone) swelling in the presence of FO

Films of poly(ε-caprolactone) were obtained by squeezing with a hydraulic press for 5 min at 5 ton. Each film was exactly weighed and than immersed in sufficient volume (2 mL) of FO in different flasks (n = 3). The flasks were closed and stored at room temperature. At predetermined time intervals (5, 12, 16, 30 and 90 days), the films were pinched and FO was removed using absorbing paper, and then the films were weighed again (Weiss-Angeli et al., 2008).

2.4. Preparation of haloperidol-loaded polysorbate-coated nanocapsules suspensions

Nanocapsules were prepared by interfacial deposition of preformed polymer, as described by Fessi et al. (1989). Briefly, the organic solution consisted of CCO, FO or GSO, poly(ε-caprolactone), haloperidol, sorbitan monostearate and acetone, which were poured under magnetic stirring into an aqueous solution containing polysorbate 80. Acetone was removed and the suspension concentrated by evaporation under reduced pressure. The final volume of the suspension was adjusted to 25 mL (0.25 mg/mL of haloperidol). Blank nanocapsules were prepared using the same protocol described above, but omitting the presence of the drug. The free suspension of haloperidol (0.25 mg/mL) was prepared in an aqueous solution using 5% (w/v) of polysorbate 80.

2.5. Physicochemical characterization of nanocapsules

Particle sizes, polydispersity indices and zeta potential (n=3) were measured by photon correlation spectroscopy (Zetasizer Nanoseries ZEN 3600, Malvern Instruments, UK). Drug

content (mg/mL) was determined (n=3) after dissolution of nanocapsules in acetonitrile (1 mL of suspension to 5 mL of acetonitrile) and assayed by high performance liquid chromatography (HPLC), according to previously described and validated protocol (Benvegnú et al., 2011) using a Shimadzu instrument (LC-10AVP Pump, UV-VIS SPD-10AVP Module, Class-VP Software, Shimadzu, Tokyo, Japan). Encapsulation efficiency was determined by the ultrafiltration-centrifugation technique. Then, the concentration of free drug was determined in the clear supernatant after this technique (Ultrafree-MC® 10,000 MW, Millipore, Bedford, USA). Encapsulation efficiency (%) was calculated by the difference between total and free drug concentrations determined in the nanocapsule suspension (drug content) and in the ultrafiltrate, respectively, using the HPLC method described above. Values of pH were determined using a calibrated potentiometer (MPA-210 Model, MS-Tecnopon, São Paulo, Brazil).

2.6. Fatty acids of nanocapsules composition

Oil samples were submitted to saponification in methanolic KOH solution and esterification in methanolic H_2SO_4 solution (Hartman and Lago, 1973). Methylated FA were analyzed using an Agilent Technologies gas chromatograph (HP 6890) equipped with a Supelco SP-2560 capillary column (100 m x 0.25 mm x 0.20 μm) and flame ionization detector. The temperature of the injector port was set at 250°C and the carrier gas was nitrogen (1.0 ml/min). After injection (1 μL, split ratio 50:1), the oven temperature was kept at 70°C for 4 min, raised to 180°C at 9°C/min and held at this temperature for 2 min, raised to 200°C at 7°C/min and held at this temperature for 5 min, raised to 210°C at 1°C/min and held at this temperature for 5 min, raised to 215°C at 1°C/min and held at this temperature for 2 min, and then raised to 240°C at 10°C/min and held at this temperature for 2.5 min. Standard FA methyl esters (Sigma, Saint Louis, USA) were subjected to the same conditions and the following retention times were used to identify the FA. Results were expressed as percentage of total area of the identified FA.

2.7. In vivo experiments

2.7.1. Experiment 1

Fifty-six rats were randomly divided in 8 groups (n=7): C group: control - aqueous solution containing 5% polysorbate 80 (v/v) – 1.0 mg/Kg, B-NcCCO group: standard blank nanocapsules suspension – 1.0 mg/Kg, FH groups: free haloperidol suspension -0.5, 0.75 and 1.0 mg/Kg; H-NcCCO groups: haloperidol-loaded standard nanocapsules suspension - 0.5, 0.75 and

1.0 mg/Kg. Animals received a daily injection ip of the treatments for 28 days. Development of oral dyskinesia (OD) was evaluated acutely (one hour after the first administration) and subchronically on days 7, 14, 21 and 28 (after the first day administration), according to the methodology described below:

2.7.1.1. Oral dyskinesia

Animals were individually placed in cages (20x20x19 cm^3) containing one mirror under the floor and one behind the back wall of the cage to allow behavioral quantification when the animal was facing away from the observer. To quantify the occurrence of OD, the incidence of vacuous chewing movement (VCM) was recorded for three sets of 5 minutes with intervals of 5 minutes. Observers were blind to the treatment. In a preliminary study (using five control and ten rats treated with haloperidol) of interrater reliability, we found that the use of this method of observation and definition for the parameters evaluated usually results in 96% agreement between three different observers.

2.7.2. Experiment 2

Fifty-six rats were randomly divided in 8 groups (n=7): C group: control-aqueous solution containing 5% polysorbate 80 (v/v), FH group: free haloperidol suspension, B-NcCCO and H-NcCCO group: blank and haloperidol-loaded nanocapsules suspension containing standard oil, B-NcFO and H-NcFO group: blank and haloperidol-loaded nanocapsules suspension containing fish oil, B-NcGSO and H-NcGSO group: blank and haloperidol-loaded nanocapsules suspension containing grape seed oil.

All formulations were administered daily (0.5 mg/kg ip) for 28 days. Development of OD was evaluated acutely and subchronically as described above.

At day 29, animals were euthanized by cervical decapitation after anesthesia with sodium pentobarbital (50 mg/kg body weight-ip). Brains were removed and cut coronally at the caudal border of the olfactory tubercle. The cortex and substantia nigra regions were dissected out (Paxinos and Watson, 2007) and homogenized in 10 vol (w/v) of 0.1 M Tris-HCl, pH 7.4, centrifuged at 1300 g (10 min) and the supernatants were used for biochemical analysis.

2.7.2.1. Slices Viability

MTT assay is a mean of measuring the activity of living cells by assessing the activity of mitochondrial dehydrogenases. The viability was quantified by measuring the reduction of [3-(4,5-dimethyllthiazol-2-yl)-2,5-diphenyltetrazolium bromide]-MTT to a dark violet formazan product by mitochondrial dehydrogenases (Mosmann, 1983). Slices (0.4 mm) of the

brain areas of rats were prepared with a Mcllwain chopper. MTT reduction assays were performed in plates containing 500 μL of phosphate buffer saline, and the reaction was started by adding MTT to a final concentration of 0.1 mg/mL. After 1 h of incubation at 37°C, the medium was removed and the slices dissolved in dimethylsulfoxide (DMSO). The MTT reduction was measured spectrophotometrically by the difference in absorbance between 570 and 630 nm. Data were calculated as a percentage of values from control.

2.7.2.2. Oxidation assay

Reactive oxygen/nitrogen species production was measured following Lebel et al. (1992) (method based on 2′7′-dichlorofluorscein (H_2DCF) oxidation. Samples (30 μL) were incubated for 30 min at 37 °C in the dark with 30 μL of 20 mM sodium phosphate buffer pH 7.4 with 140 mM KCl and 240 μL of 100 μM 2′7′-dichlorofluorscein diacetate (H_2DCF-DA) solution in a 96 wells plate. H_2DCF-DA is cleaved by cellular esterases and H2DCF formed is eventually oxidized by reactive oxygen species (ROS) or reactive nitric species (RNS) presenting in samples. The last reaction produces the fluorescent compound DCF which was measured at 488 nm excitation and 525 nm emission and the results were represented by nmol DCF/mg protein.

2.7. Statistical Analysis

Data were analyzed by one-way ANOVA followed by Duncan's multiple range analysis when required. Paired samples T-test was used to compare behavior at different time points. Pearson's correlation coefficient was calculated between VCM frequency and % cell viability determined in substantia nigra. Data were analyzed using Statistica (11.0 version) and expressed as mean±S.E.M. Significance was considered when $P<0.05$.

3. Results

Poly(ε-caprolactone) swelling in the presence of FO is shown in Figure 1.

The results showed that polymeric weights practically did not change in mass (< 1%) throughout the experimental period (day 0 to day 90).

Physico-chemical characteristics of polymeric nanocapsules are shown in Table 1 and 2.

All formulations appeared macroscopically homogeneous and their aspects were similar to a milky bluish opalescent fluid (Tyndall effect). Both blank and haloperidol

nanocapsules suspensions (NcCCO, NcFO and NcSO) presented particles within the submicrometric range (between 200 and 300 nm), low polydispersity (≤ 0.25), negative zeta potentials and neutral pH values (Table 1). The drug content was near to theorical value (0.25 mg/mL) for all suspensions. The encapsulation efficiency was excellent for H-NcCCO and H-NcFO, but low for H-NcGSO (86%) (Table 2).

The total oil content of the nanocapsules is shown in Table 3

Considerable variations of the FA composition were observed across the different oils used to prepare haloperidol-loaded nanocapsules. While in CCO the prevalence was saturated fatty acids (SFA), in FO and GSO a high proportion of monounsaturated fatty acids (MUFA) and PUFA was detected. Also, FO presented higher n-3 FA and GSO higher n-6 and n-9 FA.

Oral dyskinesia induced by acute and subchronic haloperidol treatment (free haloperidol X haloperidol-loaded nanocapsules containing caprylic/capric triglycerides*) is shown in Figure 2 and Table 4, respectively.*

Duncan post hoc test showed that rats acutely treated with FH (all doses) showed higher VCM frequency than control and H-NcCCO groups. Animals treated with the two highest doses of H-NcCCO (0.75 and 1.0 mg/kg) showed higher VCM frequency than control and B-NcCCO groups. In fact, both B-NcCCO and the lowest dose of H-NcCCO (0.5 mg/Kg) were associated with absence of oral movements, similarly to controls (Figure 2).

After subchronic treatment, the three doses of the FH group presented higher frequency of VCM than control and B-NcCCO groups did at all observation times. At the same doses of the free drug, H-NcCCO reduced OD, as observed by lower VCM frequency at days 7 (0.75 mg/kg), 14 (all doses), 21 (0.5 mg/kg) and 28 (for all doses) (Table 4). The dose of 0.5 mg/Kg of haloperidol-loaded nanocapsules suspension (H-NcCCO) was the most effective in preventing the development of OD, and therefore was chosen to be used in the following experiments.

Oral dyskinesia induced by acute and subchronic haloperidol treatment (free haloperidol and haloperidol-loaded nanocapsules containing different oils) are shown in Figure 3 and Table 5.

Duncan post hoc test showed that rats acutely treated with FH showed higher VCM frequency than controls and rats treated with blank nanocapsules (B-NcCCO, B-NcFO and B-NcGSO) and haloperidol-loaded nanocapsule suspensions containing different oils (H-

NcCCO, H-NcFO and H-NcGSO). Among these, no difference of VCM frequency was observed, but rats treated with H-NcGSO showed significantly higher VCM than control and blank nanocapsules (Figure 3) groups.

After subchronic treatment, FH administration increased VCM frequency in relation to control and to groups treated with blank nanocapsules and haloperidol-loaded nanocapsules prepared with different oils at all observation times. Among nanoencapsulated formulations, H-NcFO showed lower VCM frequency than H-NcCCO at day14 and than both HNcFO and HNcGSO at day 28^{h}, respectively. In fact, the control, blank, H-NcFO and H-NcGSO groups showed similar VCM frequency at all observation times (Table 5).

Survival of brain cells after subchronic administration of haloperidol (free and nanoencapsulated in different oils) is shown in Figure 4 and 5.

Duncan post hoc test showed that subchronic administration of FH and H-NcCCO reduced cortex cell survival as compared to the control group, while in H-NcFO and H-NcGSO groups cell survival was similar to control and blank nanocapsules groups. Except for the H-NcFO group, all haloperidol formulations (FH, H-NcCCO and H-NcGSO) reduced cell survival in substantia nigra as compared to controls, but in the H-NcGSO group this effect was smaller than in the FH group (Figure 4). Interestingly, linear regression analysis showed a significant negative correlation between VCM frequency and cell viability determined in substantia nigra (r= -0.39 and P=0.006), showing a causal relationship between damages to extrapyramidal areas and development of haloperidol-induced motor disturbances (Figure 5).

Reactive species production after subchronic administration of haloperidol (free and nanoencapsulated in different oils) is shown in Figure 6.

The production of reactive species in cortex was higher in rats treated with FH, H-NcCCO and H-NcFO than in the control group. Between haloperidol-loaded nanocapsules groups, reactive species production was lower in H-NcFO and H-NcGSO than in FH and H-NcCCO groups, whose values were similar between each other, while H-NcGSO treatment was associated with lower production of reactive species as compared to the H-NcFO group. In substantia nigra only FH increased the generation of reactive species in relation to control. In this brain area, all haloperidol-loaded nanocapsules formulations were associated with decreased production of reactive species as compared to free drug, while reactive species generation was lower in both H-NcFO and H-NcGSO groups than in the H-NcCCO group (Figure 6).

4. Discussion

In a recent study we reported that haloperidol-loaded lipid-core nanocapsules presented a stronger and most prolonged antipsychotic effect as well a reduction of motor side effects (Benvegnú et al., 2011) and OS biomarkers in relation to free drug (Benvegnú et al., 2012). As a continuation of that study, here we prepared different formulations of haloperidol-loaded nanocapsules containing caprylic/capric triglyceride, FO and GSO as core in order to compare them concerning the development of OD –a side effect commonly related to neuroleptic drug treatment – as well as their relation with free radical production and cell viability in brain areas closely involved with movement function.

A selective delivery of bioactives to restricted areas of the body in order to maximize their therapeutic potential and minimize their side-effects has been the purpose of renowned laboratories. Polymeric nanoparticles have assisted in this regard, emerging as a promising tool in drug delivery (Nahar et al., 2006). In addition, it was reported that nanoparticles overcoated by polysorbates (especially polysorbate 80) were able to transport the loaded drugs across the BBB (Schaffazick et al., 2008; Bernardi et al., 2009), suggesting their use as an alternative system to drug delivery to the brain.

Considering that the type of oily phase used as the core in preparation of polymeric nanocapsules can modify the system's properties (Bouchemal et al., 2004), we prepared nanocapsules formulations containing different oils in their core. With a similar intention, Dhanikula et al. (2007) showed the effects of a preparation of haloperidol-loaded nanocapsules containing soybean and sunflower oils. Although these oils showed some advantages, they are not considered protective *per se*, and in a way this was the motivation behind the present study. In this sense, FO was chosen because of its recognized beneficial effects in the CNS (Wainwright, 2002, Barcellos et al. 2010; 2011), while GSO was selected because of a previous study involving its incorporation in nanocapsules core (Almeida et al., 2009), as well as its antioxidant activity (Baydar et al., 2007), also favorable to the CNS.

As so far only our previous study about FO as nanocapsules core has been reported in the literature (Benvegnú et al., 2012), we performed an experiment of poly(ε-caprolactone) swelling in the presence of this oil ir order to determine a possible solubilization of the polymer by FO. In this case, the polymeric wall of nanocapsules could be dissolved by the oily core resulting in the formation of nanometric oily droplets (nanoemulsion) during the storage time (Weiss-Angeli et al., 2008). We detected just an insignificant variation in the polymeric mass, indicating that FO and poly(ε-caprolactone) have low interactions at the

macroscopic scale. Therefore, we found that the polymeric wall of nanocapsules will not be dissolved by FO, thus maintaining the required characteristics.

Haloperidol was efficiently encapsulated in polymeric nanocapsules (Fessi et al., 1989). The incorporation of FO and GSO did not interfere with the system, proving to be favorable and compatible with haloperidol and the proposed methodology. All suspensions of haloperidol-loaded nanocapsules presented mean size lower than 300 nm and drug content near 100% of the theoretical value (0.25 mg/mL). These values are in agreement with the diameters usually observed for nanocapsules prepared using the preformed polymers by the interfacial deposition method (Couvreur et al., 2002; Santos-Magalhães et al., 2000; Schaffazick et al., 2003). Polydispersity indices below 0.25 indicate a homogeneous particle distribution for all formulations (Milão et al., 2003; Alves et al., 2007). The magnitude of zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential, then they will tend to repel each other and there will be no tendency for the particles to come together (Hunter, 1981). As the formulations with GSO and FO presented higher magnitudes of zeta potential than the CCO did (~ -16, -13 and -8mV, respectively), they are less likely to be unstable. However, the physical colloidal stability of these nanocapsules also depends on the steric effect of the surfactant (polysorbate 80) at the particle/water interface (Couvreur et al., 2002). Suspensions containing GSO presented a decrease in the efficiency of haloperidol encapsulation in relation to suspension containing CCO and FO, however the mechanism involved in this effect was not established.

The composition of FA in the oils was also investigated. As expected, CCO presented only SFA (+ 99%), while GSO and FO presented mostly PUFA. Our results are according to the literature, which indicates a higher proportion of n-3 FA in FO (Stansby et al, 1969) and elevated content of n-6 FA in GSO (Baydar et al., 2007). In addition, we found a high percentage of n-9 FA in this last oil, which may contribute to its antioxidant property.

As previously demonstrated, free haloperidol at the dose of 2.0 mg/Kg-IP induced OD, while haloperidol loaded-nanocapsules were able to prevent this effect at the same dose (Benvegnú et al., 2011). In order to check if this effect is maintained with higher doses of this last formulation, we performed an experiment to compare free haloperidol with haloperidol-loaded nanocapsules at three different doses (0.5; 0.75 and 1.0 mg/Kg) which were acutely and subchronically quantified by OD. Behavioral observations showed a beneficial effect of 0.5 mg/Kg of this nanoformulation, which was able to prevent the development of OD in both acute and subchronical administration. So this dose was chosen to be used in the next

experimental set, when haloperidol-loaded nanocapsules were prepared with different oils in their core.

Acute administration of haloperidol-loaded nanocapsules containing GSO prevented OD only partially as compared to the free drug. However, subchronical administration of all nanocapsules formulations prevented this haloperidol-induced effect, showing that haloperidol-loaded nanocapsules have advantages over the free drug in its continuous use. As previously described, we hypothesized that haloperidol loaded-nanocapsules reached the extrapyramidal area more slowly and/or in lower levels, thus minimizing development of OD (Benvegnú et al., 2011). The partial prevention effect observed with acute administration of the GSO formulation may be explained by its lower encapsulation efficiency, with more drug remaining in free form. However, as this effect disappeared subchronically, the lower encapsulation efficiency did not indicate a disadvantage for this formulation.

The relationship between motor disorders and haloperidol-induced OS generation and neurotoxicity was previously described (Andreassen and Jorgensen, 2000; Tsai et al., 1998; Burger et al., 2005a). A negative correlation between development of OD and cell survival in substantia nigra confirmed the connection between haloperidol-induced neurotoxicity and development of extrapyramidal motor disorders. Increased free radical generation has been shown after prolonged haloperidol administration, which occurs by DA autooxidation and deamination (Andreassen and Jorgensen, 2000). In fact, metabolites generated in these reactions are highly reactive and can be proapoptotic, activating mitochondrial death pathways (Ukay et al., 2004). So, our findings are in accordance with these reports, mainly because, as expected, FH was able to increase ROS generation, as evidenced by DCF oxidation and reduced cell survival. These data suggest the development of apoptotic processes induced by free haloperidol, but further studies concerning apoptosis markers involved in cell death are needed.

Haloperidol-loaded nanocapsules suspension containing capric/caprilic triglycerides (H-NcCCO) was not able to prevent reduced cell survival, and did not reduce the generation of free radicals in brain areas involved in motor disorders. However, formulations containing FO and GSO (H-NcFO and H-NcGSO, respectively) had better results than H-NcCCO, which were sufficient to afford higher protection against haloperidol-induced oxidative damages. Although the H-NcGSO formulation did not show ideal physicochemical properties, it had neuroprotective effects that were better than those observed with the H-NcCCO formulation. Thus, we can suggest that nanoformulations containing PUFA and in minor proportion SFA in their core are able to enhance the therapeutic activities of the drug they carry, especially

when the active compound exerts prooxidative effects, such as haloperidol. In fact, PUFA, whose benefits to the CNS are well established, may contribute to antiapoptotic effects by controlling free radical generation and thus preventing generation of OS in the brain (Barcelos et al, 2010; 2011) and preserving neuronal survival (Murat et al., 2008; Ozlem et al., 2011). Interestinlgly, the present study is showing for the first time the beneficial effects of GSO on oxidative damages to the CNS, which may be related to its antioxidant properties (Baydar et al., 2007). Thus, haloperidol-nanocapsules suspensions containing FO (rich in n-3 FA) and GSO (rich in n-6 and n-9 FA) showed decreased generation of oxidative damages and reduced cell death, and consequently fewer drug-related adverse side effects. In general, haloperidol-loaded nanocapsules containing FO showed more adequate physicochemical parameters and fewer haloperidol-induced motor side effects, thus maintaining the functional integrity of the brain.

5. Conclusion

In summary, our study showed the beneficial effects of haloperidol-loaded nanocapsules containing different oils in their core. To the best of our knowledge, this is the first pharmacological study demonstrating the protective effects of these innovative formulations. Haloperidol-loaded nanocapsules containing FO afforded greater protection against acute and subchronic development of OD, also being able to preserve cell survival, which may be related to lower free radical generation in extrapyramidal brain areas.

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Conflict of interest

None.

References

Almeida JS, Jezur L, Fontana MC, Paese K, Silva CB, Pohlmann AR, et al. (2009). Oil-based nanoparticles containing alternative vegetable oils (grape seed oil and almond kernel oil): preparation and characterization. Acta Farm Bonaer 28:165-172.

Almeida JS, Lima F, Ros S, Bulhões LOS**,** Carvalho LM, Beck RCR (2010). Nanostructured systems containing rutin: in vitro antioxidant activity and photostability studies. Nanoscale Res Lett 5:1603-1610.

Alves MP, Scarrone AL, Santos M, Pohlmann AR, Guterres SS (2007). Human skin penetration and distribution of nimesulide from hydrophilic gels containing nanocarriers. Int J Pharm 341:215-220.

Andreassen OA, Jorgensen HA (2000). Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats - Implications for tardive dyskinesia? Prog Neurobiol 61:525-541.

Barcelos RCS, Benvegnú DM, Boufleur N, Reckziegel P, Muller L, Pase C, et al. (2010). Effects of omega-3 essential fatty acids (omega-3 EFAs) on motor disorders and memory dysfunction typical neuroleptic-induced: behavioral and biochemical parameter. Neurotox Res 17:228-237.

Barcelos RCS, Benvegnú DM, Boufleur N, Pase C, Teixeira AM, Reckziegel P, et al. (2011). Short Term Dietary Fish Oil Supplementation Improves Motor Deficiencies Related to Reserpine-Induced Parkinsonism in Rats. Lipids 46:143-149.

Baydar NG, Özkan G, Çetin ES (2007). Characterization of grape seed and pomace oil extracts. Grasas Aceites, 58:29–33.

Bazan NG (2006). The onset of brain injury and neurodegeneration triggers the synthesis of docosanoid neuroprotective signaling. Cell Mol Neurobiol 26:899–911.

Bazan NG (2007). Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. Clinl Nutr Metab Care 10:136–141.

Beck RCR, Pohlmann AR, Benvenutti EV, Costa TC, Guterres, SS (2005). Nanostructure-coated diclofenac-loaded microparticles: Preparation, morphological characterization, in vitro release and in vivo gastrointestinal tolerance. J Braz Chem Soc 16:1233-1240.

Benvegnú DM, Barcelos RCS, Boufleur N, Reckziegel P, Pase CS, Ourique AF, et al. (2011). Haloperidol-loaded polysorbate-coated polymeric nanocapsules increase its efficacy in the antipsychotic treatment in rats. Eur J Pharm Biopharm 77:332-336.

Benvegnú DM, Barcelos RCS, Boufleur N, Pase CS, Reckziegel P, Flores FC, et al. (2012). Haloperidol-loaded polysorbate-coated polymeric nanocapsules decrease its adverse motor side effects and oxidative stress markers in rats. Neurochem Int, in press, doi: 10.1016/j.neuint.2012.06.015.

Bernardi A, Braganhol E, Jager E, Figueiró F, Edelweiss MI, Pohlmann AR, et al. (2009). Indomethacin-loaded nanocapsules treatment reduces in vivo glioblastoma growth in a rat glioma model. Cancer Lett 281:53-63.

Bouchemal K, Briançon S, Perrier E, Fessi H (2004). Synthesis e characterization of polyurethane e poly (ether urethane) nanocapsules using a new technique of interfacial polycondensation combined to spontaneous emulsification. Int J Pharm 280:241-514.

Burger ME, Fachinetto R, Alves A, Callegari L, Rocha JBT (2005a). Acute reserpine and subchronic haloperidol treatment change synaptosomal brain glutamate uptake and elicid orofacial dyskinesia in rats. Brain Res 1031:202-210.

Burger ME, Rocha JBT, Fachinetto R, Callegari L (2005b). Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain. Pharmacol Biochem Behav 81:608-615.

Burger ME, Rocha JBT, Fachinetto R, Zeni G, Perottoni J, Wagner C, et al. (2006). Effects of diphenyl-diselenide on orofacial dyskinesia model in rats. Brain Res Bull 70:165-170.

Busanello A, Barbosa NBV, Peroza LR, Farias LE, Burger ME, Barreto KP, et al. (2011). Resveratrol protects against a model of vacuous chewing movements induced by reserpine in mice. Behav Pharmacol 22:71-75.

Colpo G, Trevizol F, Teixeira AM, Pereira RP, Rocha JBT, Burger ME (2007). Ilex paraguariensis has antioxidant potential and attenuates haloperidol-induced orofacial dyskinesia and memory dysfunction in rats. Neurotox Res 12:1-10.

Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C (2002). Nanocapsule technology: a review. Crit Rev Ther Drug Carrier Syst 19:99–134.

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

Dhanikula AB, Khalid NM, Lee SD, Yeung R, Risovic V, Wasan KM, et al. (2007). Long circulating lipid nanocapsules for drug detoxification. Biomaterials 28:1248-1257.

Fachinetto R, Villarinho JG, Wagner C, Pereira RP, Avila, DS, Burger ME, et al. (2007). *Valeriana officinalis* does not alter the orofacial dyskinesia induced by haloperidol in rats: Role of dopamine transporter. Prog Neuropsychopharmacol Biol Psychiatry 31:1478-1486.

Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S (1989). Nanocapsule formation by interfacial polymer deposition following solvent displacement, Int J Pharm, 55:r1–r4.

Flores FC, Ribeiro RF, Ourique AF, Beck RCR, Rolim C, Guterres SS, et al. (2011). Nanostructured systems containing an essential oil: protection against volatilization. Quím Nova 34:968-972.

Fontana MC, Rezer JFP, Coradini K, Leal DBR, Beck RCR (2011). Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. Eur J Pharm Biopharm 79:241-249.

Friese A, Seiller E, Quack G, Lorenz B, Kreuter J (2000). Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system. Eur J Pharm Biopharm 49:103–109.

Frozza RL, Bernardi A, Paese K, Hoppe JB, Silva T, Battastini AM, et al. (2010). Characterization of trans-resveratrol-loaded lipid-core nanocapsules and tissue distribution studies in rats. J Biomed Nanotechnol 6:694-703.

Gao Y, Xu P, Chen L, Li Y (2010). Prostaglandin E_1 encapsulated into lipid nanoparticles improves its anti-inflammatory effect with low side-effect. Int J Pharm 387:263-271.

Hartman L, Lago BC (1973). A rapid preparation of fatty methyl esters from lipids. Lab. Pract. 22, 475–477.

Hunter RJ (1981). Zeta potential in colloid science: principles and applications. Academic Press: London.

Ianiski FR, Alves CB, Souza AC, Pinton S, Roman SS, Rhoden CR, et al. (2012). Protective effect of meloxicam-loaded nanocapsules against amyloid-β peptide-induced damage in mice. Behav Brain Res 230:100-107.

Lebel CP, Ischiropoulos H, Bondy SC (1992). Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol, 5:227–231.

Milão D, Knorst MT, Guterres SS (2003). Hydrophilic gel containing nanocapsules of diclofenac: development, stability study and physico-chemical characterization. Pharmazie 58:325–329.

Mitchell IJ, Cooper AC, Griffiths MR, Cooper AJ (2002). Acute administration of haloperidol induces apoptosis of neurons in the striatum and substantia nigra in the rat. Neuroscience 109:89–99.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. J Immunol Methods, 65:55–63.

Murat C, Ahmet S, Onder S, Efkan U, Ramazan Y, Murat Y, et al (2008). The neuroprotective effect of fish n-3 fatty acids in the hippocampus of diabetic rats. Nutr Neurosci 11:161-166.

Muthu MS, Rawat MK, Mishra A, Singh S (2009). PLGA nanoparticle formulations of risperidone: preparation and neuropharmacological evaluation. Nanomedicine, 5:323-333.

Nagarwal RC, Kant S, Singh PN, Maiti P, Pandit JK (2009). Polymeric nanoparticulate system: A potential approach for ocular drug delivery. J Control Release, 136:2-13.

Nahar M, Dutta T, Murugesan S, Asthana A, Mishra D, Rajkumar V, et al (2006). Functional polymeric nanoparticles: an efficient and promising tool for active delivery of bioactives. Crit Rev Ther Drug Carrier Syst 23:259–318.

Ourique AF, Melero A, Silva CB, Schaefer UF, Pohlmann AR, Guterres SS, et al. (2011). Improved photostability and reduced skin permeation of tretinoin: Development of a semisolid nanomedicine. Eur J Pharm Biopharm 79:95-101.

Ozlem O, Yasemin SC, Gulay H, Yargicoglu P, Demir R, Agar A, et al (2011). The influence and the mechanism of docosahexaenoic acid on a mouse model of Parkinson's disease. Neurochem Int 59:664-670.

Parikh T, Bommana MM, Squillante E (2010). Efficacy of surface charge in targeting pegylated nanoparticles of sulpiride to the brain. Eur J Pharm Biopharm 74:442-450.

Paxinos G, Watson C (2007). The rat brain in stereotaxic coordinates, sixth ed. Elsevier:Amsterdam.

Ponto T, Ismail NI, Majeed ABA, Marmaya NH, Zakaria ZA (2010). A prospective study on the pattern of medication use for schizophrenia in the outpatient pharmacy department, Hospital Tengku Ampuan Rahimah, Selangor, Malaysia. Methods Meth Find Exp Clin Pharmacol 32:427-432.

Santos-Magalhães NS, Pontes A, Pereira VMW, Caetano MNP (2000). Caetano, Colloidal carriers for benzathine penicillin G: Nanoemulsions and nanocapsules. Int J Pharm 208:71-80.

Schaffazick SR, Guterres SS, Lucca-Freitas L, Pohlmann AR (2003). Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos. Quím Nova 26:726–737.

Schaffazick SR, Siqueira IR, Badejo AS, Jornada DS, Pohlmann AR, Netto CA, et al. (2008). Incorporation in polymeric nanocapsules improves the antioxidante effect of melatonin against lipid peroxidation in mice brain and liver. Eur J Pharm Biopharm, 69:64–71.

Seju U, Kumar A, Sawant KK (2011). Development and evaluation of olanzapine-loaded PLGA nanoparticles for nose-to-brain delivery: In vitro and in vivo studies. Acta Biomater 7:4169-4176.

Stansby ME (1969) Nutritional properties of fish oils. World Rev Nutr Diet, 11:46-105.

Trevizol F, Benvegnú DM, Barcelos RCS, Pase CS, Segat H, Dias VT, et al. (2011). Comparative study between two animal models of extrapyramidal movement disorders: prevention and reversion by pecan nut shell aqueous extract. Behav Brain Res 221:13-18.

Tsai G, Goff DC, Change RW, Flood J, Bair L, Coyle JT (1998). Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. Am J Psych 155:1207.

Ukai W, Ozawa H, Tateno M, Hashimoto E, Saito T (2004). Neurotoxic potential of haloperidol in comparison with risperidone. Implication of Aktmediated signal changes by haloperidol. J Neural Transm 111:667–681.

Wainwright PE (2002). Dietary essential fatty acids and brain function: a development perspective on mechanisms. Proceed Nutr Soc, 61:61–69.

Weiss-Angeli V, Polleto FS, Zancan LR, Baldasso F, Pohlmann AR, Guterres SS (2008). Nanocapsules of octyl methoxycinnamate containing quercetin delayed the photodegradation of both components under ultraviolet A radiation. J Biom Nanotechnol 4, 80-89.

Wohlfart S, Khalansky AS, Gelperina S, Begley D, Kreuter J (2011). Kinetics of transport of doxorubicin bound to nanoparticles across the blood–brain barrier. J Control Release 154:103–107.

Yen FL, Wu TH, Lin LT, Cham TM, Lin CC (2008). Nanoparticles formulation of Cuscuta chinensis prevents acetaminophen-induced hepatotoxicity in rats. Food Chem Toxicol 46:1771-1777.

Zararsiz I, Kus I, Akpolat N, et al. (2006). Protective effects of omega-3 essential fatty acids against formaldehyde induced neuronal damage in prefrontal cortex of rats. Cell Biochem Funct 24:237–244.

Zhu BT (2004). CNS dopamine oxidation and catechol-O-methyltransferase: Importance in the etiology, pharmacotherapy, and dietary prevention of Parkinson's disease. Int J Mol Med 13:343-353.

Zhu L, Ma J, Jia N, Zhao Y Shen H (2009). Chitosan-coated magnetic nanoparticles as carriers of 5-Fluorouracil: Preparation, characterization and cytotoxicity studies. Colloids Surf B Biointerfaces 68:1-6.

Figure Captions

Figure 1. Poly(ε-caprolactone) swelling experiments in presence of fish oil. Films were weighed at different time intervals.

Figure 2. Acute effects of different doses of a single administration of free and nanoencapsulated haloperidol on vacuous chewing movements (VCM) frequency in rats. Abbreviations: C-control; B-NcCCO and H-NcCCO (blank- and haloperidol-loaded nanocapsules containing caprylic/capric triglycerides; FH-free haloperidol. Data are expressed as mean±S.E.M (n=7). *Indicates significant difference from C group; $^{+}$Indicates significant difference from FH group (P<0.05 for all comparisons).

Figure 3. Acute effects of a single administration (0.5 mg/kg/mL-ip) of free and nanoencapsulated haloperidol containing different oils as core on vacuous chewing movements (VCM) frequency in rats. Abbreviations: C (control); B-NcCCO and H-NcCCO (blank and haloperidol-loaded nanocapsules containing caprylic/capric triglycerides), B-NcFO and H-NcFO (blank and haloperidol-loaded nanocapsules containing fish oil); B-NcGSO and H-NcGSO (blank and haloperidol-loaded nanocapsules containing grape seed oil); FH-free haloperidol. Data are expressed as mean±S.E.M. (n=7). *Indicates significant difference from C group; $^{+}$Indicates significant difference from FH group (P<0.05 for all comparisons).

Figure 4. Subchronic effects of free or nanoencapsulated haloperidol containing different oils (0.5 mg/Kg/mL- ip once a day, for 28 days) on % cell viability measured by MTT assay in cortex (A) and substantia nigra (B) of rats. Abbreviations: C (control); B-NcCCO and H-NcCCO (blank and haloperidol-loaded nanocapsules containing caprylic/capric triglycerides), B-NcFO and H-NcFO (blank and haloperidol-loaded nanocapsules containing fish oil); B-NcGSO and H-NcGSO (blank and haloperidol-loaded nanocapsules containing grape seed oil); FH-free haloperidol. Data are expressed as mean±S.E.M. (n=7). *Indicates significant difference from C group; $^{+}$Indicates significant difference from FH group; $^{\#}$Indicates significant difference from H-NcCCO group (P<0.05 for all comparisons).

Figure 5. Linear regression analysis between % substantia nigra cell viability and VCM frequency in rats treated with free or nanoencapsulated haloperidol containing different oils

(0.5 mg/Kg/mL- ip once a day, for 28 days, n=7). Linear regression was evidenced by Pearson's correlation coefficients (r = -0.39 and P<0.05).

Figure 6. Subchronic effects of free or nanoencapsulated haloperidol containing different oils (0.5 mg/Kg/mL- ip once a day, for 28 days) on the production of reactive species measured by DCF assay in cortex (A) and substantia nigra (B) of rats. Abbreviations: C (control); B-NcCCO and H-NcCCO (blank and haloperidol-loaded nanocapsules containing caprylic/capric triglycerides), B-NcFO and H-NcFO (blank and haloperidol-loaded nanocapsules containing fish oil); B-NcGSO and H-NcGSO (blank and haloperidol-loaded nanocapsules containing grape seed oil); FH-free haloperidol. Data are expressed as mean±S.E.M. (n=7). *Indicates significant difference from C group; $^{+}$Indicates significant difference from FH group; $^{\#}$Indicates significant difference from H-NcCCO group (P<0.05 for all comparisons); ^{o}Indicates significant difference from H-NcFO group (P<0.05 for all comparisons).

Figure 1:

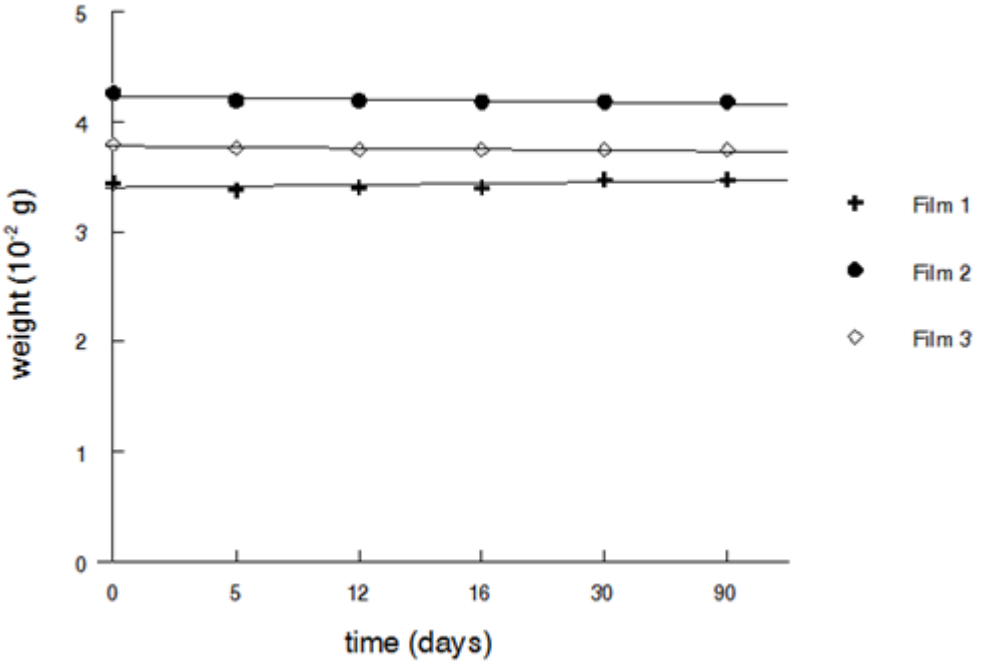


Table 1: Physicochemical characteristics of blank and haloperidol loaded-nanocapsules.

Nanocapsules	Particle size (nm)	PDI[a] (mv)	Zeta Potential	pH
B-NcCCO	280±15	0.25±0.1	-8±0.5	7.1±0.2
B-NcFO	290±14	0.23±0.0	-12±1.6	7.0±0.2
B-NcGSO	272±25	0.24±0.0	-16±2.1	7.2±0.2
H-NcCCO	230±25	0.16±0.5	-8±0.2	7.5±0.1
H-NcFO	261±03	0.21±0.0	-13±0.7	7.5±0.1
H-NcGSO	253±30	0.20±0.0	-16±1.0	7.4±0.2

Mean±S.D.: represents the variation among the different batches (n=3).
[a] PDI: polydispersity index.

Table 2: Drug content and encapsulation efficiency of blank and haloperidol loaded-nanocapsules.

Nanocapsules	Drug Content (mg/mL)	Encapsulation efficiency (%)
H-NcCCO	0.25±0.2	94±0.5
H-NcFO	0.25±0.0	95±0.4
H-NcGSO	0.25±0.1	86±0.3

Mean±S.D.: represents the variation among the different batches (n=3).

Table 3: Fatty acid composition of nanocapsules oil content (% of total oil identified).

Fatty acids	CCO	FO	GSO
8:0	54.05	0.16	0.01
10:0	45.44	0.16	0.04
14:0	0.01	7.54	0.07
16:0	0.06	19.23	6.42
18:0	0.11	4.21	3.95
22:0	nd	0.16	0.76
∑ SFA	99.67	31.45	11.25
16:1 n-7	nd	9.31	0.10
18:1 n-9	0.02	12.50	33.36
20:1 n-9	nd	1.25	0.24
∑ MUFA	0.02	23.06	33.70
18:2 n-6	nd	1.87	53.76
18:3 n-3	nd	1.03	0.29
20:2 n-6	nd	3.39	0.02
20:4 n-6	nd	1.04	nd
20:5 n-3	nd	18.86	nd
22:5 n-3	nd	2.63	0.02
22:6 n-3	nd	13.20	nd
∑ PUFA	nd	42.02	54.09
∑ n-3	nd	35.72	0.31
∑ n-6	nd	6.30	53.78
∑ n-9	0.02	13.75	33.6

The following fatty acids were found at concentrations lower than 0.5% and for this reason are not shown: C4:0, C6:0, C11:0, C12:0, C13:0, C15:0, C17:0, C20:0, C18:3n6, C20:3n6, C20:3n3, C24:0 and C24:1n9. The following fatty acids were not detected in the analyzed samples: C14:1n5, C15:1n5, C17:1n5, C18:1n9t, C18:2n6t, C21:0, C20:3n3, C22:1n9, C23:0 and C22:2n6. SFA-saturated fatty acids; MUFA-monounsaturated fatty acids; PUFA-polyunsaturated fatty acids; nd-not detected.

Figure 2:

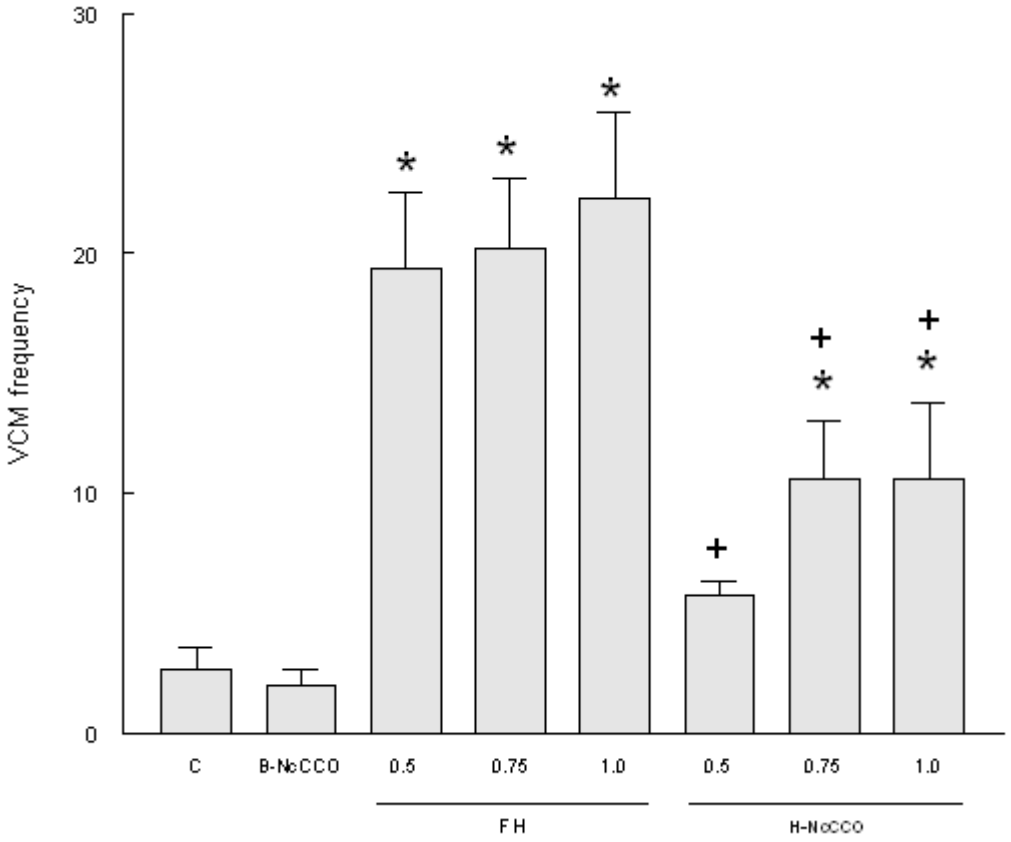


Table 4: Table 4. Subchronic effects of daily administration of different doses of free or nanoencapsulated haloperidol for 28 days on vacuous chewing movement (VCM) frequency in rats.

Groups	Days			
	7	14	21	28
C	4.25±1.44	3.42±1.70	4.43±1.53	2.37±0.96
B-NcCCO	5.83±2.30	5.25±2.75	5.33±2.27	5.58±4.53
FH				
0.5	23.14±11.4*	20.73±8.36*	18.90±4.99*	16.14±3.51*
0.75	15.80±2.74*	22.00±5.86*	17.00±4.04*	26.90±2.62*
1.0	25.67±8.10*	32.25±6.85*	27.25±3.74*	32.20±10.11*
H-NcCCO				
0.5	8.50±1.60	5.75±1.98^{+}	6.80±1.93^{+}	7.50±2.17^{+}
0.75	4.83±1.56^{+}	2.77±0.79^{+}	9.58±2.95	6.67±3.62^{+}
1.0	20.202±4.00*	17.83±4.26*$^{+}$	26.58±5.61*	20.33±4.55*$^{+}$

These observations were performed at 7, 14, 21 and 28 days after the first injection. Abbreviations: C (control); B-NcCCO and H-NcCCO (blank and haloperidol-loaded nanocapsules containing caprylic/capric triglycerides), B-NcFO and H-NcFO (blank and haloperidol-loaded nanocapsules containing fish oil); B-NcGSO and H-NcGSO (blank and haloperidol-loaded nanocapsules containing grape seed oil); FH-free haloperidol. Data are expressed as mean±S.E.M (n=7). *Indicates significant difference from C group; $^{+}$Indicates significant difference from FH group in the same dose (P<0.05 for all comparisons).

Figure 3:

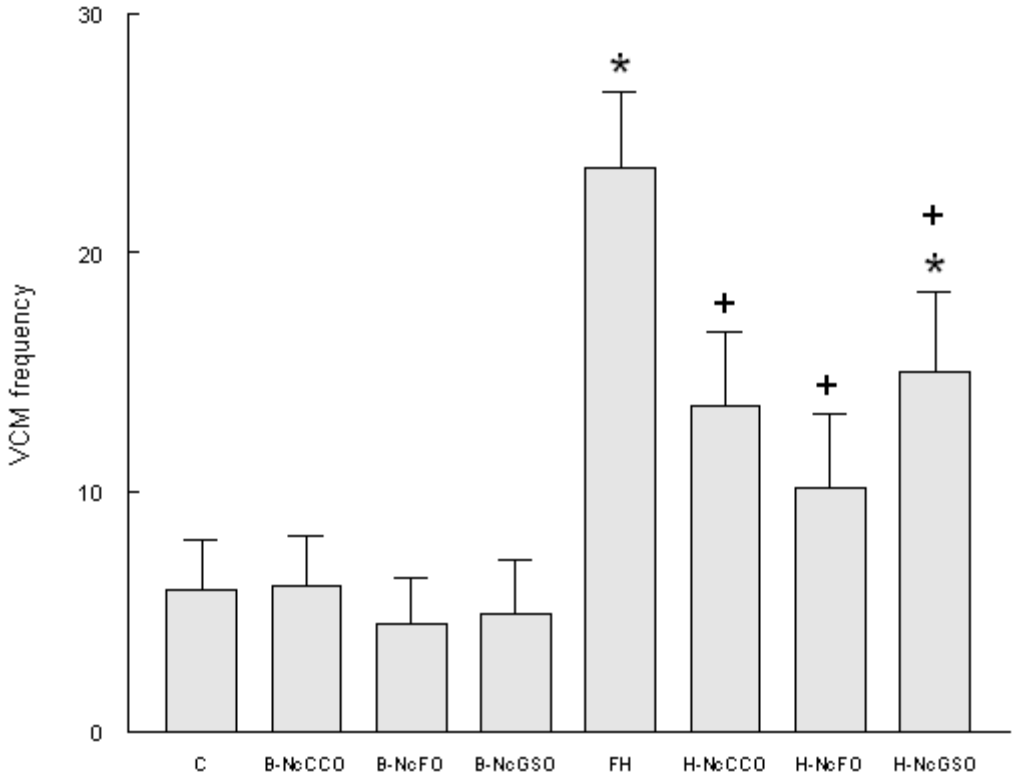

30
20
10
0
VCM frequency
*
+
+
+
*
C
B-NcCCO
B-NcFO
B-NcGSO
FH
H-NcCCO
H-NcFO
H-NcGSO

Table 5: Subchronic effects of daily administration of free or nanoencapsulated haloperidol containing different oils (0.5 mg/Kg/mL-IP once a day, for 28 days) on vacuous chewing movement (VCM) frequency in rats.

Groups	Days			
	7	14	21	28
C	5.29±1.24	7.85±1.49	3.93±1.95	5.91±2.28
B-NcCCO	6.3±1.76	1.71±0.58	3.14±0.81	2.21±0.67
B-NcFO	5.64±1.48	2.85±1.55	3.21±1.21	1.92±0.70
B-NcGSO	6.8±3.09	2.1±0.94	11.5±3.43	1.50±0.41
FH	22.6±6.07*	21.87±2.90*	22.65±4.97*	21.00±3.53*
H-NcCCO	13.78±4.34	$10.57\pm3.47^{+}$	$10.06\pm2.87^{+}$	$11.14\pm62.63^{+}$
H-NcFO	$6.94\pm0.67^{+}$	$4.00\pm1.47^{+\#}$	$4.06\pm1.71^{+}$	$3.08\pm0.81^{+\#}$
H-NcGSO	$10.42\pm1.69^{+}$	$6.00\pm1.34^{+}$	$6.78\pm3.11^{+}$	$3.93\pm1.08^{+\#}$

These observations were performed at 7, 14, 21 and 28 days after the first injection. Abbreviations: C (control); B-NcCCO and H-NcCCO (blank and haloperidol-loaded nanocapsules containing caprylic/capric triglycerides), B-NcFO and H-NcFO (blank and haloperidol-loaded nanocapsules containing fish oil); B-NcGSO and H-NcGSO (blank and haloperidol-loaded nanocapsules containing grape seed oil); FH-free haloperidol. Data are expressed as mean±S.E.M. (n=7). *Indicates significant difference from C group; $^{+}$Indicates significant difference from FH group; $^{\#}$Indicates significant difference from H-NcSO group ($P<0.05$ for all comparisons).

Figure 4:

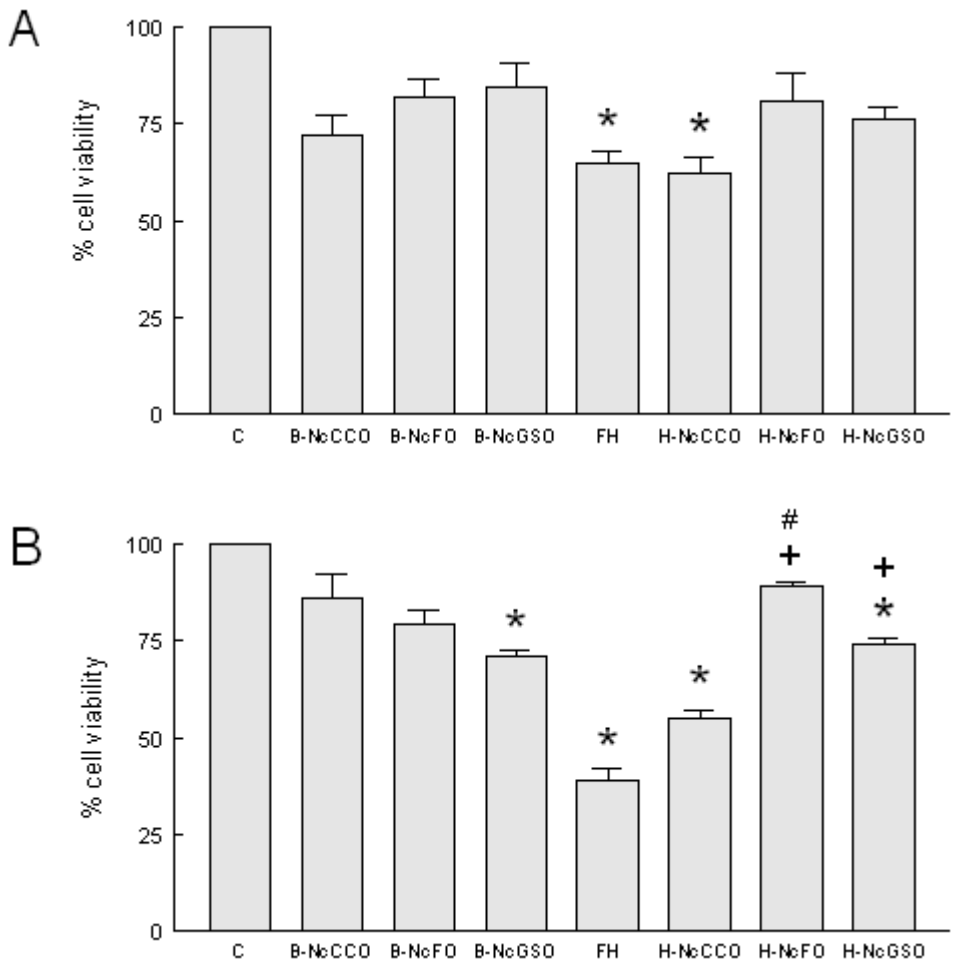

A
% cell viability
100
75
50
25
0
C
B-NcCCO
B-NcFO
B-NcGSO
FH
H-NcCCO
H-NcFO
H-NcGSO
*
*
B
% cell viability
100
75
50
25
0
C
B-NcCCO
B-NcFO
B-NcGSO
FH
H-NcCCO
H-NcFO
H-NcGSO
*
*
*
#
+
+
*

Figure 5:

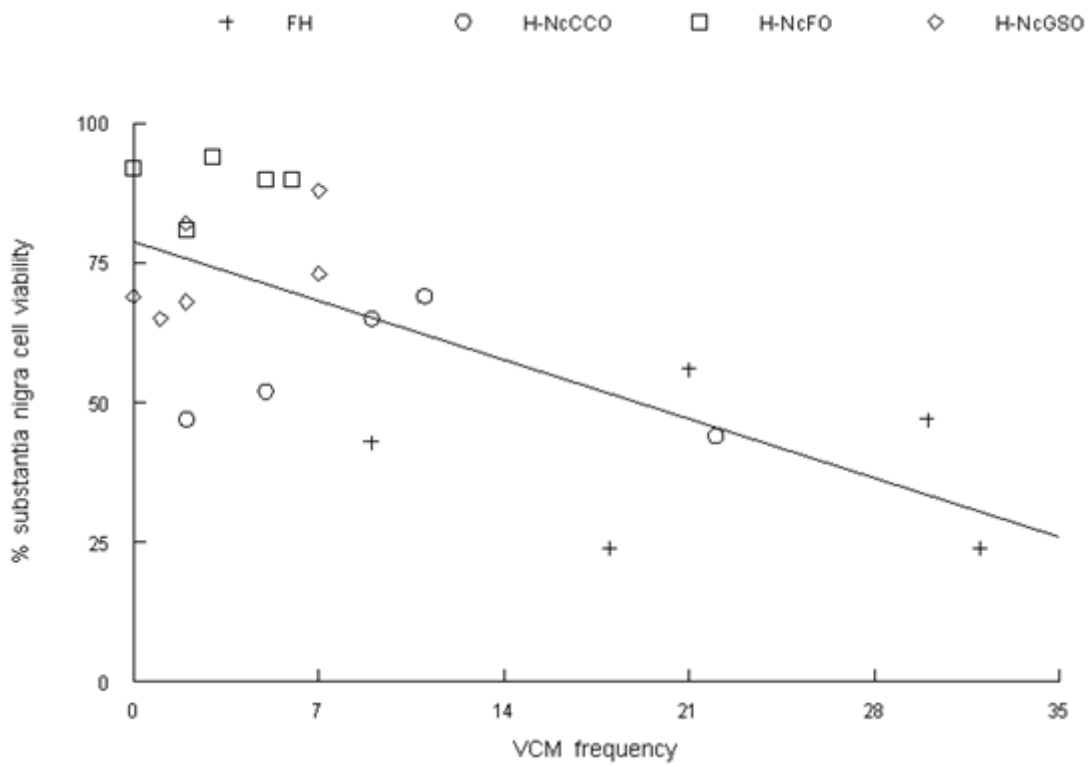
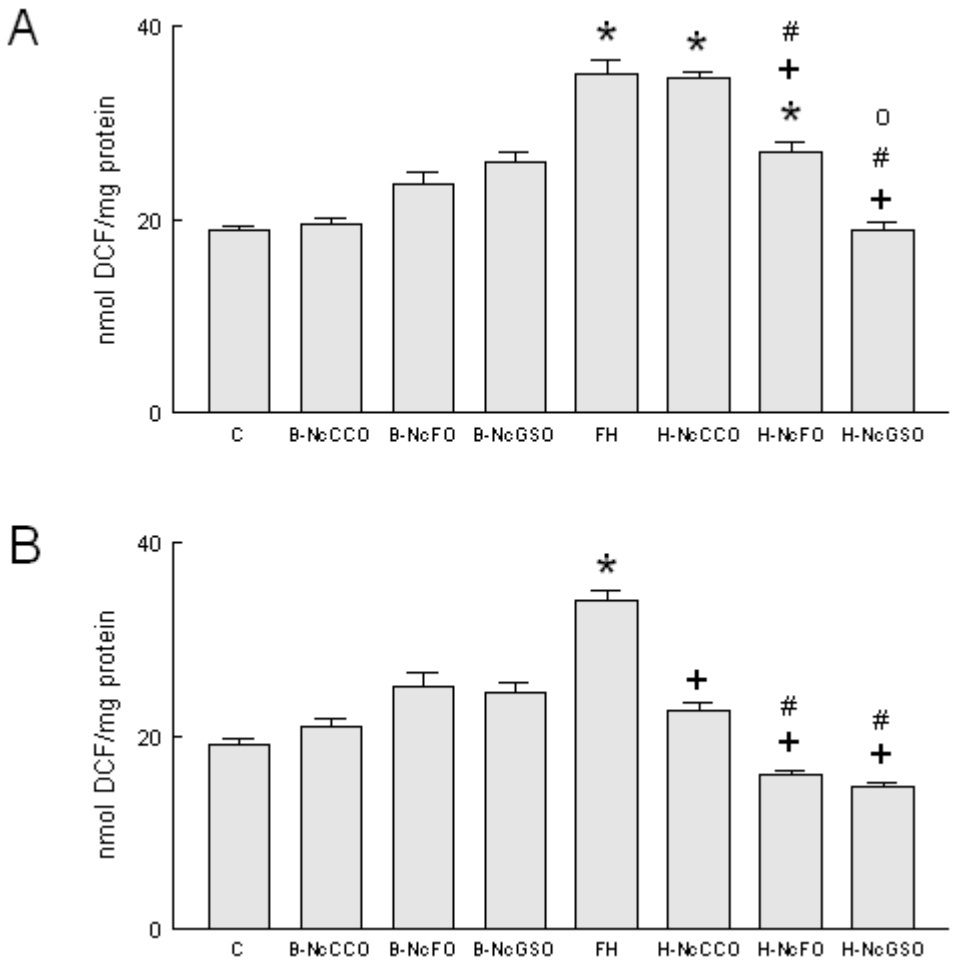

FH
H-NcCCO
H-NcFO
H-NcGSO
% substantia nigra cell viability
100
75
50
25
0
0
7
14
21
28
35
VCM frequency

Figure 6:


A
nmol DCF/mg protein
40
20
0
*
*
#
+
*
o
#
+
C
B-NcCCO
B-NcFO
B-NcGSO
FH
H-NcCCO
H-NcFO
H-NcGSO
B
nmol DCF/mg protein
40
20
0
*
+
#
+
#
+
C
B-NcCCO
B-NcFO
B-NcGSO
FH
H-NcCCO
H-NcFO
H-NcGSO

4 DISCUSSÃO

O haloperidol continua sendo o fármaco antipsicótico mais prescrito mundialmente (SILVA, 2002; PONTO et al., 2010), o qual é classificado como antagonista dopaminérgico predominantemente D_2 (FANG et al., 1997), cuja ação terapêutica relaciona-se ao bloqueio das vias dopaminérgicas mesolímbica e mesocortical (CREESE et al., 1976). No entanto, o antagonismo das demais vias dopaminérgicas acaba gerando graves efeitos adversos, os quais podem ser progressivos e incapacitantes, comprometendo, assim, a qualidade de vida dos pacientes que necessitam desta medicação (MORENO et al., 2004).

Nos últimos anos, muitos esforços do nosso grupo de pesquisa foram investidos em busca da prevenção ou redução dos efeitos colaterais motores induzidos por antipsicóticos típicos. Estes estudos tiveram por base a utilização de substâncias com propriedades antioxidantes, a fim de reduzir os danos oxidativos associados ao uso de antipsicóticos, cuja fisiopatologia têm sido relacionada ao EO e à neurotoxicidade. Como retrospectiva, entre os antioxidantes estudados, encontram-se alguns compostos sintéticos (BURGER et al., 2003; 2005b; BURGER et al., 2006), compostos naturais como o óleo de peixe (BARCELOS et al., 2010) e o resveratrol (BUSANELLO et al., 2011) ou, ainda, plantas medicinais de reconhecida atividade antioxidante, tais como *Valeriana officinalis* (FACHINETTO et al., 2007), *Ilex paraguariensis* (COLPO et al., 2007) e *Carya illinoensis* (TREVIZOL et al., 2011). Além disso, passou-se a investigar outras formas de prevenção, como a atividade física regular, que foi capaz de prevenir os distúrbios motores induzidos pelo haloperidol (TEIXEIRA et al., 2011), bem como desordens do movimento associadas ao processo natural de envelhecimento (TEIXEIRA et al., 2012).

Neste sentido, na busca pela minimização de efeitos adversos dos fármacos, destaca-se a nanotecnologia. No âmbito farmacêutico, os sistemas carreadores nanoestruturados possibilitam uma diminuição da dose e, consequentemente, dos efeitos adversos, visto que aumentam o controle da liberação, a especificidade e a seletividade ao local de ação do fármaco, proporcionando, assim, um aumento da eficácia terapêutica (COUVREUR & VAUTHIER, 2006). Desta forma, fármacos que estavam obsoletos pelo fato de apresentarem maiores malefícios que benefícios podem, com isto, serem novamente reavaliados.

Diante das inúmeras vantagens que a nanotecnologia farmacêutica tende a oferecer, a hipótese do presente trabalho é de que as nanocápsulas poliméricas poderiam ser capazes de modificar o direcionamento e/ou a liberação do haloperidol, favorecendo o aumento da

eficácia farmacológica, bem como a diminuição dos efeitos colaterais em relação ao fármaco livre.

Assim, com o objetivo de desvendar a hipótese proposta, verificou-se a possibilidade de associar o haloperidol a um sistema nanoestruturado. Pelo fato deste fármaco ser muito lipossolúvel, optou-se pela formulação de nanocápsulas poliméricas, além de que o invólucro polimérico seria capaz de auxiliar no controle da liberação (LANGER, 1993). O polímero utilizado foi a PCL, devido às suas propriedades de biocompatibilidade e biodegradabilidade (SINHA et al., 2004). O método de preparação escolhido foi o da deposição interfacial do polímero pré-formado, proposto por Fessi et al. (1989), já que é um método fácil, rápido, eficaz e tradicionalmente utilizado na preparação de nanocápsulas. Com isto, demonstrou-se que o haloperidol é um fármaco capaz de ser nanoencapsulado, visto que não foi observada nenhuma incompatibilidade ou inadequação, tal como agregação de partículas ou precipitados, durante ou ao término da preparação das suspensões. Assim, macroscopicamente, as formulações apresentaram aspecto homogêneo, semelhante a um fluido opalescente branco leitoso, com um reflexo azulado frente à observação contra a luz. Este reflexo é conhecido como efeito *Tyndal* e é devido ao tamanho nanométrico e ao movimento Browniano das partículas (MAGENHEIM & BENITA, 1991).

A fim de observar se o haloperidol havia sido nanoencapsulado de forma satisfatória, realizou-se a caracterização físico-química das suspensões desenvolvidas. Foi verificado um tamanho nanométrico, dentro da faixa esperada para nanopartículas poliméricas preparadas pelo método de nanoprecipitação (COUVREUR et al., 2002. SCHAFFAZICK et al., 2003). O índice de polidispersão abaixo de 0,25 indica uma distribuição homogêna das partículas. O potencial zeta negativo, com elevado valor em módulo reflete a estabilidade das suspensões, mostrando a repulsão entre as partículas, evitando, assim, uma possível agregação e tendência à precipitação (COUVREUR et al., 2002). Além disso, o teor do fármaco foi próximo a 100% e a eficiência de encapsulação foi de 95%, mostrando resultados satisfatórios. Tomados todos estes resultados em conjunto, demonstrou-se a viabilidade das suspensões contendo nanocápsulas poliméricas de haloperidol.

Com a finalidade de estudar a eficácia terapêutica das suspensões descritas acima, procedeu-se com a escolha de um modelo animal adequado. Vários são os modelos de indução de pseudo-psicose descritos na literatura, como o modelo da apomorfina (VÕIKAR et al., 1999; MOTAMAN et al., 2005), fenciclidina (SAMS-DODD, 1998a; 1998b; DUNN; KILLCROSS, 2006), cetamina (SHIIGI; CASEY, 1999; BECKER; GRECKSCH, 2004), cocaína (BUDYGIN, 2007; CORTEZ et al., 2010) e anfetamina (SAMS-DODD, 1998b;

BATTISTI et al., 2000; WOLGIN; JAKUBOW, 2004). Optou-se por este último modelo, por ser bem representativo e difundido. De acordo com Robinson & Becker (1986) e Lyon (1991), a anfetamina é capaz de promover um conjunto de movimentos estereotipados em animais, semelhantes aos sintomas positivos da esquizofrenia observados em humanos. Desta forma, a D-anfetamina é comumente utilizada como modelo animal de indução de psicose (CAMP; ROBINSON, 1988; DICKINSON et al., 1988; ROFFMAN; RASKIN, 1997; SAMS-DODD, 1998b). A partir do modelo eleito, escolheu-se dois métodos de análise que consistem na observação de movimentos estereotipados através de escala de escores: o método proposto por Cresse e Yversen (1973), que tem por base o monitoramento dos movimentos corporais; e o método descrito por Ujike et al. (1990), que quantifica os movimentos de cabeça.

Devido ao fato de se dispor apenas da D,L-anfetamina, forma racêmica, foi necessária a padronização e validação metodológica para essa substância. De acordo com a literatura, a dose de D-anfetamina capaz de induzir movimentos estereotipados por via intraperitoneal varia normalmente na faixa de 2 a 6mg/Kg (FUENMAYOR; DIAZ, 1984; DICKINSON et al., 1988; COLE; KOOB, 1989; MOORE; KENYON, 1994; ROFFMAN; RASKIN, 1997; KUCZENSKI; SEGAL, 1999). Assim, ao utilizar uma anfetamina racêmica, foram escolhidas doses acima desta faixa, em proporcionalidade, a fim de ser obtido um efeito semelhante. Desta forma, foi realizada uma curva nas doses de 4, 6, 8, 12 e 16 mg/Kg-IP. As doses de 4 e 6 mg/Kg mostraram um aumento da atividade locomotora; entretanto, os movimentos estereotipados não foram tão característicos ou iniciaram de forma tardia. Já as doses de 12 e 16 mg/Kg induziram movimentos estereotipados muito drasticamente. Sendo assim, foi escolhida a dose de 8 mg/Kg, por demonstrar com clareza os movimentos estereotipados, tanto de corpo quanto de cabeça e por esses aumentarem de forma progressiva no decorrer no tempo.

Após definida a dose, foi verificado o tempo de duração do efeito da anfetamina e a necessidade de novas administrações. Assim, verificou-se que uma hora após a adminitração ocorria um pico na escala de estereotipia, sendo que esse efeito poderia aumentar ou se manter durante a segunda hora. No entanto, a partir da terceira hora, observou-se um declínio na intensidade dos movimentos estereotipados. Desta forma, foi estabelecido que, a cada três horas, uma nova dose de anfetamina deveria ser administrada.

Em relação à dose do fármaco, foram estudadas diversas doses de haloperidol na sua forma livre e nanoencapsulada, as quais deveriam ser capazes de demonstrar efeito antipsicótico frente ao modelo da anfetamina, bem como diferir no tempo e na eficácia terapêutica. Ou seja, o objetivo consistiu em determinar qual a dose do fármaco em que

poderia ser vista uma diferença de efeito entre o haloperidol livre e o nanoencapsulado. Assim, partiu-se da dose de 1 mg/Kg, dose terapêutica, na qual não foi observada diferença entre as formulações. Posteriormente, de forma sucessiva, as doses foram sendo diminuídas, até obtenção da dose ideal, 0,2 mg/Kg, na qual o haloperidol nanoencapsulado demonstrou melhor efeito na redução dos movimentos estereotipados em comparação ao fármaco livre. Após isto, objetivou-se responder a seguinte questão: o haloperidol nanoencapsulado seria capaz de manter uma eficácia antipsicótica por um período de tempo mais prolongado? Para tal, foi realizado um experimento no decorrer do tempo. A partir da nona hora após a administração da anfetamina, ou seja, após a quarta administração, foi verifcado que a formulação de haloperidol livre não demonstrou mais efeito antipsicótico e que a formulação de haloperidol nanoencapsulado foi capaz de manter esse efeito por pelo menos até a décima segunda hora, tempo final de experimentação.

Os resultados do presente trabalho estão de acordo com os achados de Muthu et al., (2009), que mostrou uma melhor eficácia antipsicótica da risperidona quando associada a um sistema nanoparticulado polimérico, frente ao modelo de pseudo-psicose induzido por apomorfina em camundongos. Além disso, outros estudos em nível de SNC foram capazes de demonstrar um aumento da eficácia terapêutica de fármacos, utilizando-se sistemas compostos por nanocápsulas poliméricas (SCHAFFAZICK et al., 2008; WU et al., 2008; BERNARDI et al., 2009, XU et al., 2009). Desta forma, pode-se sugerir que o aumento da eficácia terapêutica pode ser devido a um maior aporte cerebral do fármaco ou devido a um direcionamento para o síto de ação, como demonstrado em outros trabalhos (BERNARDI et al., 2008; KUO; LIANG, 2011). Com respeito ao prolongamento do tempo de ação, já está estabelecido na literatura que as nanocápsulas tendem a desempenhar um papel importante, modificando a liberação do fármaco e possibilitando que essa liberação seja mais controlada e mais sustentada (BECK et al., 2005; 2006; 2007; FONTANA et al., 2009; MARCHIORI et al., 2010), diferentemente do que ocorre com o fármaco livre, cuja liberação se dá de forma imediata.

Uma vez verificada a eficácia da formulação proposta, prosseguiu-se com o estudo dos efeitos colaterais motores, os mais preocupantes, visto que são incapacitantes e que comprometem a qualidade de vida dos pacientes esquizofrênicos que fazem uso do haloperidol, sendo dado um enfoque especial neste sentido.

A fim de representar o que ocorre com os pacientes que desenvolvem a síndrome da DT, após uso de antipsicóticos ou mesmo apenas alguns dos efeitos extrapiramidais decorrentes do bloqueio D_2 no sistema nigro-estriatal (CREESE et al., 1976), avaliou-se o

desenvolvimento da DO, através da contagem dos movimentos de mascar no vazio (MMV) e o comportamento cataléptico, por meio da verificação do tempo de imobilidade em modelos animais. Como foi utilizada a dose de 0,2 mg/Kg-IP de haloperidol para o experimento da eficácia terapêutica, essa mesma dose foi mantida, inclusive para o estudo dos efeitos colaterais motores, os quais foram analisados agudamente (1 hora após administração do fármaco) e subcronicamente (28 dias após a administração do fármaco).

Após a experimentação, observou-se que a formulação de haloperidol nanoencapsulado foi capaz de prevenir ou minimizar os efeitos colaterais motores, tanto de forma aguda quanto subcrônica, demonstrando mais uma vantagem em relação ao fármaco livre. Os achados novamente estão de acordo com os resultados obtidos por Muthu et al. (2009), os quais demonstraram uma diminuição do comportamento cataléptico de camundongos após tratamento com risperidona veiculada a um sistema nanoparticulado. Mais estudos corroboram com os dados da pesquisa, mostrando que as nanopartículas são capazes de diminuir os efeitos adversos sistêmicos de fármacos em geral (BECK et al., 2005; YEN et al., 2008; GAO et al., 2010) e fármacos utilizados para tratamento de doenças referentes ao SNC (BERNARDI et al., 2009; WONG et al., 2010; WOHLFART et al., 2011; IANISKI et al., 2012). Assim, a prevenção ou minimização dos efeitos adversos observados pode ocorrer devido a modificações na liberação, visto que uma liberação mais controlada e não imediata dificultaria picos de acúmulo do fármaco em sítios de ação não específicos. Além disso, poderia ser cogitada a possibilidade de um direcionamento do fármaco para o seu local de ação, ou seja, uma vetorização para a via dopaminérgica mesolímbica, reduzindo a chegada de fármaco na via dopaminérgica nigro-estriatal. No entanto, os mecanismos que poderiam levar a este efeito seriam desconhecidos. Como, de forma concomitante, à diminuição dos efeitos adversos foi observado um aumento da eficácia antipsicótica, poderia ser levada em conta a possibilidade de uma redução na dose de administração do haloperidol, quando na forma nanoencapsulada, favorecendo ainda mais a diminuição dos efeitos colaterais motores.

Desta forma, tomados em conjunto os dados, demonstrou-se, de forma inédita, os benefícios do haloperidol nanoencapsulado, formulação que foi capaz de apresentar uma maior eficácia terapêutica assim como uma menor geração de efeitos colaterais motores em relação ao fármaco livre.

Na sequência deste estudo, novas suspensões de nanocápsulas de haloperidol foram preparadas com óleo de peixe, em substituição ao óleo contendo TCM, mais comumente empregado, com a finalidade de verificar se a modificação deste núcleo oleoso seria capaz de contribuir positivamente com a resposta biológica. A razão para esta substituição teve por

base resultados promissores observados com o óleo de peixe sobre distúrbios do movimento, recentemente obtidos pelo grupo de pesquisa (BARCELOS et al, 2010; 2011; TEIXEIRA et al., 2011; 2012).

Desta forma, tomou-se por base o haloperidol nanoencapsulado, o qual demonstrou aumento da eficácia terapêutica e diminuição dos efeitos colaterais motores. Substituiu-se o núcleo oleoso composto por TCM pelo óleo de peixe, cuja preparação seguiu o mesmo protocolo. Ao final, foi obtida uma formulação com aspecto branco leitoso, homogêneo e com reflexo azulado, conforme esperado. A caracterização físico-química mostrou-se satisfatória, visto que o tamanho das partículas ficou dentro da faixa nanométrica; o índice de polidispersão abaixo de 0,25, mostrando homogeneidade entre as partículas e potencial zeta negativo, com elevado valor em módulo, indicando estabilidade. Além disso, o teor e a taxa de encapsulação do fármaco foram de 99,6% e 95%, respectivamente, demonstrando adequabilidade da formulação. Para esta formulação, foi realizado, ainda um estudo morfológico das partículas, via microscopia eletrônica de transmissão, cujo resultado confirmou o tamanho nanométrico, bem como a forma esférica e uniforme das partículas.

Em relação ao estudo em animais, inicialmente realizou-se um experimento piloto para verificar se a nova formulação seria capaz de apresentar uma eficácia superior à formulação contendo TCM como núcleo oleoso. Utilizou-se, assim, o mesmo modelo de pseudo-psicose induzida por anfetamina, seguindo o método previamente padronizado. Os achados demonstraram que a eficácia terapêutica da formulação de haloperidol nanoencapsulado contendo óleo de peixe foi semelhante à das nanocápsulas de haloperidol contendo TCM. Desta forma, os dados obtidos não foram incluídos em nenhum manuscrito e passou-se a se deter apenas ao estudo dos efeitos colaterais motores da formulação.

Assim, o próximo objetivo referente à formulação de óleo de peixe foi a determinação da intensidade dos efeitos adversos motores induzidos pelo haloperidol e a relação com marcadores de EO nas regiões cerebrais envolvidas no movimento. Novamente teve-se por base o estudo realizado por Barcelos et al. (2010) envolvendo o óleo de peixe e parâmetros motores e de EO verificados após a administração de haloperidol.

Para tal, realizou-se um experimento em ratos com a formulação de haloperidol livre e nanoencapsulado contendo óleo de peixe, na mesma dose (0,2 mg/Kg-IP) dos experimentos anteriores, nos quais novamente foram avaliados, de forma aguda e subcrônica, os movimentos orais, como medida da indução de DO e o tempo de imobilidade, como medida do comportamento cataléptico. Além disso, verificou-se a atividade locomotora e exploratória dos animais em um campo aberto. Por fim, foram realizadas análises de marcadores de EO,

como LPO, através do método de substâncias reativas ao ácido tiobarbitúrico (TBARS), bem como defesas antioxidantes, enzimática, no caso da catalase (CAT) e não enzimática, como a glutationa reduzida (GSH) no córtex, estriado e substância negra. A fim de determinar o envolvimento do EO com os distúrbios do movimento, foi feita uma correlação entre os vários parâmetros analisados.

Os resultados demonstraram que a suspensão de nanocápsulas de haloperidol, contendo óleo de peixe, foi capaz de prevenir ou minimizar os efeitos colaterais motores, agudos e subcrônicos, demonstrando, mais uma vez, que o sistema nanoencapsulado pode ser mais vantajoso que o fármaco livre.

Em relação aos parâmetros de EO, foi observado que o fármaco livre induziu um aumento dos níveis de TBARS, bem como uma redução nos níveis de GSH e atividade da CAT nas diferentes regiões cerebrais estudadas. Já o haloperidol nanoencapsulado, contendo óleo de peixe, foi capaz de prevenir todas as alterações acima citadas. Assim, esta última formulação apresentou efeitos adversos menores, bem como foi capaz de prevenir a geração do quadro de EO.

Os achados corroboraram, novamente, com dados anteriores do grupo de pesquisa, mostrando o envolvimento do EO na geração de efeitos colaterais motores induzidos pelo haloperidol (BURGER et al., 2005a; 2005b; COLPO et al., 2007; FACHINETTO et al., 2005; BARCELOS et al., 2010, TEIXEIRA et al., 2011), visto que foi verificada uma correlação positiva entre a geração de LPO e a indução de DO e catalepsia, assim como uma correlação negativa entre a LPO e a atividade locomotora horizontal e vertical.

A relação entre EO e a fisiopatologia dos distúrbios do movimento induzidos pelo haloperidol já está bem estabelecida (TSAI et al., 1998). O efeito do metabólito tóxico HPP^+, assim como o bloqueio dos receptores D_2 pelo haloperidol, são dois fatores que podem contribuir, de forma sinérgica, para o aumento da liberação de DA (BLOOMQUIST et al., 1993; ANDREASSEN; JORGENSEN, 2000). E, como já mencionado anteriormente, o metabolismo da DA gera RL via atividade das oxidases em geral e demais reações posteriores, além do subproduto, denominado dopamina-quinona, que é reconhecido por ser citotóxico e por sua capacidade de depletar os estoques de GSH (LOHR et al., 1991; ANDREASSEN; JORGENSEN, 2000). Além disso, o bloqueio dopaminérgico estriatal pode gerar um aumento na liberação de glutamato extracelular, gerando mais RL via mecanismos de excitotoxicidade (COYLE; PUTTFARCKEN, 1993; TSAI et al., 1998, BURGER et al., 2005a).

Assim, levando-se em conta todos os achados, demonstrou-se, pela primeira vez, a viabilidade de uma formulação de haloperidol nanoencapsulado contendo óleo de peixe, a qual foi capaz de minimizar os efeitos colaterais motores desencadeados pelo processo de EO.

Tendo em vista que as suspensões de nanocápsulas de haloperidol, contendo o óleo padrão, TCM, bem como a sua substituição pelo óleo de peixe, mostraram resultados satisfatórios em nível de formulação e efeito biológico, objetivou-se assim, desenvolver uma nova formulação do fármaco, utilizando o óleo de semente de uva. No que diz respeito à escolha do óleo, primeiramente, já havia sido demonstrada a adequabilidade desse óleo no desenvolvimento de nanocápsulas (ALMEIDA et al., 2009; 2010). Além disso, foram consideradas as propriedades terapêuticas do óleo, destacando-se suas propriedades antioxidantes (BAYDAR et al., 2007) e o fato de que dados da literatura carecem de estudos acerca de possíveis benefícios deste óleo sobre o SNC.

Desta forma, foram preparadas as suspensões contendo nanocápsulas de haloperidol com óleo de semente de uva, pelo mesmo método descrito, cujas aparências macroscópicas mostraram ausência de incompatibilidades físico-químicas, bem como uniformidade, indicando viabilidade da formulação. Juntamente às nanocápsulas, contendo o óleo de semente de uva, foram preparadas nanocápsulas com TCM e com o óleo de peixe, a fim de realizar-se um estudo comparativo entre as três formulações.

Primeiramente, foram realizados estudos acerca da composição dos ácidos graxos presentes nos diferentes óleos. Conforme esperado, o óleo composto por TCM apresentou apenas ácidos graxos saturados, enquanto que o óleo de peixe e óleo de semente de uva apresentaram um teor mais elevado de ácidos graxos poliinsaturados. Além disso, os resultados estão de acordo com dados da literatura, que demonstram um predomínio de ácidos graxos da série ômega-3 no óleo de peixe (STANSBY et al, 1969; WAINWRIGHT et al., 2002), assim como um predomínio de ácidos graxos da série ômega-6, seguido pela série do ômega-9, no óleo de semente de uva (BOCKISCH, 1993; FIRESTONE, 1999; BAYDAR et al., 2007).

Após a caracterização físico-química das diferentes suspensões, verificou-se que as formulações apresentaram partículas homogêneas, visto que o índice de polidispersão ficou abaixo de 0.25 e tamanho nanométrico. Em relação ao potencial zeta, as formulações apresentaram valores negativos, mostrando-se estáveis. No entanto, as formulações contendo os óleos de peixe e de uva demonstraram menor tendência à instabilidadade frente à formulação contendo TCM, visto que apresentaram valores superiores em módulo. O teor do fármaco também foi satisfatório (~ 100%) para todas as suspensões. Entretanto, no que diz

respeito à taxa de encapsulação do fármaco, as formulações preparadas com o óleo de semente de uva apresentaram uma redução desse parâmetro (85%), permanecendo maior concentração de fármaco na forma livre, diferindo das demais formulações (95%).

Os experimentos realizados até então foram todos na dose de 0,2 mg/Kg-IP de fármaco, que havia sido a melhor dose para o estudo da eficácia terapêutica. Diferentemente, pensou-se em realizar um estudo com o objetivo de determinar se mesmo em doses mais elevadas o haloperidol nanoencapsulado continua sendo mais vantajoso que o fármaco livre. Para tal, foram escolhidas as doses de 0,5; 0,75 e 1 mg/Kg-IP de fármaco livre e nanoencapsulado e administradas de forma aguda e subcrônica, a fim de avaliar a indução de DO. Os dados do estudo mostraram que, de forma aguda, o haloperidol nanoencapsulado na dose de 0,5 mg/Kg não induziu DO, sendo esta parcialmente observada nas demais doses. A administração subcrônica da suspensão de nanocápsulas de haloperidol induziu DO apenas na dose de 1 mg/Kg. Desta forma, foi escolhida a dose de 0,5 mg/Kg para a continuidade dos experimentos, principalmente por não ter induzido DO aguda ou subcronicamente.

Após determinação da dose, foi realizado um estudo comparativo entre as três formulações de haloperidol nanoencapsulado, bem como o fármaco livre, com o objetivo de verificar a indução de DO aguda e subcrônica, assim como a geração de RL e a viabilidade celular nas regiões envolvidas no controle do movimento.

Os resultados obtidos demonstraram que as formulações de haloperidol nanoencapsulado, contendo TCM e óleo de peixe na dose de 0.5 mg/Kg-IP, preveniram totalmente a indução de DO agudamente; já, subcronicamente, todas as formulações foram capazes de prevenir este efeito. Em relação às nanocápsulas contendo óleo de semente de uva, o menor efeito protetor observado agudamente pode ser explicado devido à menor eficiência de encapsulação, permanecendo maior quantidade de fármaco livre.

A neurotoxicidade induzida pelo haloperidol já está bem documentada (GALILI et al., 2000; ZHURAVLIOVA et al., 2007; OZBEK et al., 2010). Conforme anteriormente mencionado, este fármaco é capaz de induzir a formação de RL, devido a um acúmulo de DA e sua consequente metabolização (ANDREASSEN; JORGENSEN, 2000). Além disso, ele pode induzir a liberação de proteínas apoptóticas pelas células, as quais podem levar à ativação de caspases, contribuindo, desta forma, para a indução de morte neuronal (UKAI et al., 2004). Os resultados do presente estudo estão de acordo com estas observações, visto que verificou-se um aumento na formação de RL, observado através da maior oxidação da dicloro-fluoresceína, assim como uma redução na viabilidade celular, evidenciada por uma inibição na redução do MTT. Este último dado poderia sugerir, indiretamente, um aumento do

processo apoptótico. No entanto, maiores estudos envolvendo marcadores apoptóticos seriam necessários para confirmar esta hipótese, visto que a determinação da viabilidade celular indica apenas a funcionalidade celular, e não exatamente, a morte celular.

Já havia sido demonstrada, no experimento anterior, uma relação entre EO e os efeitos colaterais motores induzidos pelo haloperidol. Aqui, mostrou-se uma correlação negativa entre a viabilidade celular na região da substância negra e o desenvolvimento de DO, confirmando as hipóteses já descritas, de que a neurotoxicidade oxidativa do haloperidol está intimamente relacionada ao desenvolvimento das desordens motoras (BLOOMQUIST, 1993).

A formulação de nanocápsulas de haloperidol contendo TCM não mostrou eficiência em relação à manutenção da viabilidade celular e no controle da geração de RL. Já as formulações contendo óleo de peixe e óleo de semente de uva mostraram efeitos satisfatórios, contribuindo para a manutenção da integridade e funcionalidade celular; ou seja, as formulações contendo óleos antioxidantes apresentaram uma maior proteção. Assim, pode-se sugerir que o potencial neuroprotetor destes óleos possa estar contribuindo para os efeitos benéficos observados aqui. Desta forma, demonstrou-se que o óleo de semente de uva poderia estar desempenhando um papel protetor em nível de SNC, sendo este efeito decorrente do próprio óleo, devido à presença de ácidos graxos poliinsaturados da série n-6 e n-9 e não da formulação de nanocápsulas, visto que a formulação contendo TCM, composta apenas por ácidos graxos saturados, não mostrou tal efeito. Já o óleo de peixe apresenta muitos dados na literatura acerca do papel neuroprotetor de seus ácidos graxos poliinsaturados da série n-3 (WAINWRIGHT, 2002), conferindo benefícios contra diversas desordens neurológicas e psiquiátricas, como depressão (SILVERS, et al., 2005, SINCLAIR, 2011), hiperatividade (YEHUDA et al., 2005), doença de Huntington (CLIFFORD et al., 2002), Alzheimer (COLE et al., 2005; CHIU et al., 2008; JÖNHAGEN, 2008) e Parkinson (DELATTRE et al., 2010; BOUSQUET et al., 2011), além da própria esquizofrenia (JOY et al. 2000; ARVINDAKSHAN et al., 2003; DAS, 2004; SIVRIOGLU et al., 2007).

Ainda, a suspensão de nanocápsulas de haloperidol contendo óleo de peixe mostrou uma maior adequabilidade em relação aos parâmetros físico-químicos de formulação, além de uma maior eficiência no controle dos efeitos colaterais motores induzidos pelo fármaco, mantendo a integridade funcional do cérebro. Estes achados podem ser decorrentes da excelente característica do óleo em relação à formulação, bem como à sua potencial propriedade neuroprotetora ou, ainda, a união dos dois fatores. No entanto, maiores estudos referentes à farmacocinética das nanocápsulas de haloperidol, preparadas com os diferentes óleos, seriam necessários, a fim de elucidar os achados aqui mostrados.

Assim, demonstrou-se, pela primeira vez, as vantagens da formulação de haloperidol nanoencapsulado, aumentando a eficácia terapêutica e prevenindo ou minimizando a geração de efeitos colaterais motores, relacionados ao processo de EO. Foi demonstrado, também, o desenvolvimento de suspensões de nanocápsulas de haloperidol contendo óleos alternativos, constituídos por ácidos graxos poliinsaturados, como óleo de peixe e óleo de semente de uva, sendo que a formulação contendo óleo de peixe mostrou maior eficiência no controle dos efeitos adversos, como a DO, visto que foi capaz de preservar a viabilidade celular e prevenir a geração de RL em regiões cerebrais relacionadas ao controle do movimento**.**

5 CONCLUSÕES

Através dos resultados obtidos podemos chegar às seguintes conclusões:

1. O haloperidol foi devidamente nanoencapsulado pelo método proposto, mostrando adequabilidade em relação aos parâmetros físico-químicos avaliados. Além disso, as suspensões de nanocápsulas de haloperidol contendo óleo de peixe e óleo de semente de uva mostraram-se viáveis quanto a aspectos de formulação nanométrica.

2. A formulação contendo haloperidol nanoencapsulado mostrou uma atividade terapêutica mais eficaz e mais duradoura em relação ao fármaco livre.

3. A formulação contendo haloperidol nanoencapsulado preveniu o desenvolvimento de DO e reduziu a intensidade da catalepsia, tanto de forma aguda quanto subcrônica. Em relação à atividade locomotora, a formulação contendo haloperidol nanoencapsulado não demonstrou vantagens em relação ao fármaco livre.

4. A formulação de haloperidol livre foi capaz de alterar parâmetros de EO, os quais foram correlacionados aos distúrbios do movimento. Este efeito não foi observado com a formulação de haloperidol nanoencapsulado.

5. As formulações de haloperidol nanoencapsulado contendo TCM e óleo de peixe preveniram totalmente a geração de DO aguda, ao passo que a formulação contendo óleo de semente de uva preveniu parcialmente. Já, de forma subcrônica, todas as formulações mostraram-se eficazes no sentido de prevenção.

6. As formulações de haloperidol nanoencapsulado contendo TCM não demonstraram proteção frente à manutenção da viabilidade celular e controle da geração de radicais livres. Contrariamente, as formulações de óleo de peixe e óleo de semente de uva apresentaram um efeito neuroprotetor, confirmando seus benefícios.

PERSPECTIVAS

Com base nos resultados obtidos no presente trabalho, faz-se necessário os seguintes estudos:

- ✓ Avaliação da liberação *in vitro* do haloperidol nanoencapsulado.
- ✓ Quantificação das formulações de haloperidol em nível plasmático e cerebral.
- ✓ Avaliação do efeito terapêutico e efeitos colaterais motores após a administração oral e intramuscular das formulações de haloperidol.
- ✓ Efeito do tratamento com as formulações de haloperidol sobre parâmetros cognitivos em ratos.
- ✓ Efeito do tratamento com as formulações de haloperidol sobre parâmetros de hepatotoxicidade em ratos.
- ✓ Efeito do tratamento com as formulações de haloperidol sobre parâmetros sexuais e endócrinos em ratos.
- ✓ Efeito do tratamento com as formulações de haloperidol sobre marcadores apoptóticos *in vitro* e *in vivo*.

REFERÊNCIAS BIBLIOGRÁFICAS

ABI-DARGHAM, A. Do we still believe in the dopamine hypothesis? New data bring new evidence. **Int. J. Neuropsychopharmacol.**, v.7, p.01-05, 2004.

ABBOTT, N.J. et al. Structure and function of the blood–brain barrier. **Neurobiol. Dis**., v. 37, p.13-25, 2010.

ABREU, P. B.; BOLOGNESI, G.; ROCHA, N. Prevenção e tratamento de efeitos adversos de antipsicóticos. **Rev. Bras. Psiquiatr.**, n. 22, p. 41-44, 2000.

AEBI, U.; CHIU, W.; MILLIGAN, R. Role of catalase on antioxidative defenses. **J. Struct. Biol.**, v. 2, p.117-118, 1995.

AHMAD, Z. et al., Alginate nanoparticles as antituberculosis drug carriers: formulation development, pharmacokinetics and therapeutic potential. **Indian J. Chest Dis. Allied. Sci.**, v. 48, p.171-176, 2006.

AHMAD, Z. The uses and properties of almond oil. **Complement. Ther. Clin. Pract.**, v. 16, p. 10-12, 2010.

ALBENSI, B. C. The NMDA receptor/ion channel complex: a drug target for modulating synaptic plasticity and excitotoxicity. **Curr. Pharm. Des.**, v. 13, 31, pp. 3185-3194, nov. 2007.

ALLEN, H.Y.; EVERITT, Z.M; JUDD, A.T. Haloperidol monograph for UKPID, 1998.

ALLEN, T.M.; CULLIS, P.R. Drug delivery systems: entering the mainstream. **Science**, v. 303, p.1818-1822, 2004.

ALMEIDA, J.S. et al. Oil-based nanoparticles containing alternative vegetable oils (grape seed oil and almond kernel oil): preparation and characterization. **Acta Farm. Bonaerense**, v.28, p.165-172, 2009.

ALMEIDA, J.S. et al. Nanostructured systems containing rutin: in vitro antioxidant activity and photostability studies. **Nanoscale Res. Lett.**, v.5, p.1603-1610, 2010.

ALVAREZ, G.F.; SKOWRONSKI, G.A. Remember the side effects of haloperidol: a case report. **Crit Care Resusc.,** v.5, p.266-269, 2003.

AMERICAN PSYCHIATRIC ASSOCIATION (APA). Practice guideline for the treatment of patients with schizophrenia. 2. ed. 2004.

ANANTH, J.; PARAMESWARAN, S.; HARA, B. Drug therapy in schizophrenia. **Curr. Pharm. Des.**, v.10, p.2205-2217, 2004.

ANDREASSEN, O.A.; JORGENSEN, H.A. GM1 ganglioside attenuates the development of vacuous chewing movements induced by long term haloperidol treatment of rats. **Psychopharmacol.**, v.116, p.517-522, 1994.

ANDREASSEN, O. A.; JORGENSEN, H. A. Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats: implications for tardive dyskinesia? **Prog. Neurobiol.**, v.61, p.525-541, 2000.

ARINOLA, O.G.; IDONIJE, O. B. Status of plasma nitric oxide and non-enzymatic antioxidants before and after antipsychotic treatment in Nigerian patients with schizophrenia. **J. Res. Med. Sci.**, v.14, p.37–42, 2009.

ARRUDA, E.V.; MORAIS, H.L.M.N.; PARTATA, A.K. Avaliação das informações contidas em receitas e notificações de receitas atendidas na farmácia do CAPS II Araguaína-TO. **Revista Científica do ITPAC**, v.5, p.1-14, 2012.

ARVINDAKSHAN, M. et al. Supplementation with a combination of ω-3 fatty acids and antioxidants (vitamins E and C) improves the outcome of schizophrenia . **Schizophr. Res.**, v.62, p.195-204, 2003.

ASANUMA, M.; MIYAZAKI, I.; OGAWA, N. Dopamine- or L-DOPA-induced neurotoxicity: the role of dopamine quinone formation and tyrosinase in a model of Parkinson's disease. **Neurotox. Res.**, v.5, p.165-176, 2003.

ASSISI, A. et al. Fish oil and mental health: the role of n-3 long-chain polyunsaturated fatty acids in cognitive development and neurological disorders. **Int. Clin. Psychopharmacol.**, v.21, p.319-336, 2006.

AZEVEDO, M. M. Nanoesferas e a liberação controlada de fármacos. Laboratório de Química do Estado Sólido. São Paulo: UNICAMP, 2002.

BAGCHI, D. et al. Cellular protection with proanthocyanidins derived from grape seeds. **Ann. NY Acad. Sci.**, v.957, p.260-270, 2002.

BAPTISTA, T. Body weight gain induced by antipsychotic drugs: mechanisms and management. **Acta Psychiatr. Neurol. Scand.,** v.100, p.03-16, 1999.

BARCELOS, R. C. S. et al. Effects of ω-3EssentialFattyAcids(ω-3EFAs) on motor disorders and memory dysfunction typical neuroleptic-induced: behavioral and biochemical parameter. **Neurotox. Res.**, v.17, p.228-237, 2010.

BARCELOS, R.C.S. et al. Short term dietary fish oil supplementation improves motor deficiencies related to reserpine-induced Parkinsonism in rats. **Lipids**, v. 46:143–149, 2011.

BARRATT, G. M. Therapeutic applications of colloidal drug carriers. **Pharm. Sci. Tech. Today**, v.3, p.163–71, 2000.

BATTISTI, J.J.; URETSKY, N.J.; WALLACE, L.J. Importance of environmental context in the development of amphetamine- or apomorphine-induced stereotyped behavior after single and multiple doses. **Pharmacol. Biochem. Behav.**, v. 66, p. 671-677, 2000.

BAYDAR, N.G.; ÖZKAN, G.; ÇETIN, E.S. Characterization of grape seed and pomace oil extracts. **Grasas Aceites**, v.58, p.29–33, 2007.

BEASLEY, C. M.; TOLLEFSON, G. D.; TRAN, P. V. Safety of olanzapine. **J. Clin. Psych.**, v.58, p.13- 17, 1997.

BECHELLI, L. P. C. Impacto e evolução do emprego dos neurolépticos no tratamento da esquizofrenia. **J. Bras. Psiquiatr.**, v.49, p.131-47, 2000.

BECK, R. C. R. et al. Nanostructure-coated diclofenac-loaded microparticles: preparation, morphological characterization, in vitro release and in vivo gastrointestinal tolerance. **J. Braz. Chem. Soc.**, v.16, p.1233-1240, 2005.

BECK, R. C. R. et al. Nanoparticle-coated organic-inorganic microparticles: experimental design and gastrointestinal tolerance evaluation. **Quím. Nova**, v.29, p.990-996, 2006.

BECK, R. C. R. et al. Dexamethasone-loaded nanoparticlecoated microparticles: correlation between *in vitro* drug release and drug transport across caco-2 cell monolayers. **Eur. J. Pharm. Biopharm.**, v.67, p.18-30, 2007.

BECKER, A.; GRECKSCH, G. Ketamine-induced changes in rat behaviour: a possible animal model of schizophrenia. Test of predictive validity **Prog. Neuropsychopharmacol. Biol. Psych.**, v.28, p.1267-1277, 2004.

BERESFORD, R.; WARD, A. Haloperidol decanoate: a preliminary review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in psychosis. **Drugs**, v.33, p.31-49, 1987.

BERNARDI, A. et al. Indomethacin-loaded nanocapsules treatment reduces *in vivo* glioblastoma growth in a rat glioma model. **Cancer Lett.,** v.281, p.53-63, 2009.

BERKLAND, C. et al. Microsphere size, precipitation kinetics and drug distribution control drug release from biodegradable polyanhydride microspheres. **J. Control. Release**, v. 94, p. 129-141, 2004.

BERNARDI, A.; FROZZA, R.L.; HORN, A.P. Protective effects of indomethacin-loaded nanocapsules against oxygen-glucose deprivation in organotypic hippocampal slice cultures: Involvement of neuroinflammation. **Neurochem. Int.**, v.57, p.629-636, 2010.

BLACK, K.L. et al. The protective effects of dietary fish oil on focal cerebral infarction. **Prostaglandins Med.**, v.3, p.257-568, 1979.

BLACK, K.L. et al. Eicosapentaenoic acid: effect on brain prostaglandins, cerebral blood flow and edema in ischemic gerbils. **Stroke**, v.15, p. 65-69, 1984.

BLOOMQUIST, J. et al. MPP^{+}-like neurotoxicity of a pyridinium metabolite of haloperidol. **Soc. Neurosci. Abstr.**, v.19, p.1680, 1993.

BLOOMQUIST, J. et al. MPP^{+}-like neurotoxicity of a pyridinium metabolite derived from haloperidol: cell culture and neuro-transmitter uptake studies. **J. Pharmacol. Exp. Ther.**, v.270, p.822–830, 1994.

BODOR, N.; BUCHWALD, P. Recent advances in the brain targeting of neuropharmaceuticals by chemical delivery systems. **Adv. Drug Deliv. Rev.**, v.36, p.229-254, 1999.

BOURRE, J.M. Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during aging. **J. Nutr. Health Aging**, v.8, p.163-74, 2004.

BLIN, O. A comparative review of new antipsychotics. **Can. J. Psych.,** v.44, p.235-244, 1999.

BOCKISCH, M., 1993. Nahrungsfette und O et al. On the aroma of Austrian pumpkin seed oil. Correlation of analytical data with olfactoric characteristics. Ernaehrung (Vienna), v.226, p.246–249, 1998.

BOUCHEMAL, K. et al. Syntesis e characterization of polyurethane e poly (ether urethane) nanocapsules using a new technique of interfacial polycondensation combined to spontaneous emulsification. **Int. J. Pharm.**, v.280, p.241-51, 2004.

BOUSQUET, M.; CALON, F.; CICCHETTI, F. Impact of omega-3 fatty acids in Parkinson's disease. **Ageing Res. Rev.**, v.10, p.53-463, 2011.

BOYNE, A. F.; ELLMAN, G. L. A methodology for analysis of tissue sulfhydryl components, **Anal. Biochem.**, v.46, p.639-653, 1972.

BOWIE, C.R.; HARVEY, P.D. Schizophrenia from neuropsychiatric perspective. **Mount Sinai J. Med.**, v. 73, p.993-998, 2006.

BRANDÃO, M.L.; GRAEFF, F.G. **Neurobiologia das doenças mentais**. 3 ed. São Paulo: Lemos Editorial, 1996.

BRANDÃO, A. Brasileiros revolucionam nos medicamentos microscópicos. **Pharmacia Brasileira**, ano IX, n. 47, 2005.

BRANNON-PEPPAS, L.; BLANCHETTE, J.O. Nanoparticle and targeted systems for cancer therapy. **Adv. Drug Deliv. Rev.**, v.56, p.1649–1659, 2004.

BRIGGER, I.; DUBERNET, C.; COUVREUR, P. Nanoparticles in cancer therapy and diagnosis. **Adv. Drug Del. Rev.**, v.54, p.631-651, 2002.

BROMET, E. J.; FENNING, S. Epidemiology and natural history of schizophrenia. **Biol. Psych.**, v.46, p.871-881, 1999.

BUCKLEY, P.F.; STHAL, S.M. Pharmacological treatment of negative symptoms of schizophrenia: therapeutic opportunity or Cul-de-sac? **Acta Psych. Scand.**, v.115, p.93–100, 2007.

BUDHIAN, A.; SIEGEL, S.J.; WINEY, K.I. Production of haloperidol-loaded PLGA nanoparticles for extended controlled drug release of haloperidol. **J. Microencapsul.**, v.22, p.773-785, 2005.

BUDHIAN, A.; SIEGEL, S.J.; WINEY, K.I. Haloperidol-loaded PLGA nanoparticles: Systematic study of particle size and drug content. **Int. J. Pharm.**, v.336, p.367-375, 2007.

BUDHIAN, A.; SIEGEL, S.J.; WINEY, K.I. Controlling the in vitro release profiles for a system of haloperidol-loaded PLGA nanoparticles. **Int. J. Pharm.**, v.346, p.151-159, 2008.

BUDYGIN, E.A. Dopamine uptake inhibition is positively correlated with cocaine-induced stereotyped behavior. **Neurosci. Lett.**, v.429, p. 55-58, 2007.

BURGER, M.E. et al. Ebselen attenuates reserpine-induced orofacial dyskinesia and oxidative stress in rat striatum. **Prog. Neuropsychopharmacol. Biol. Psych.**, v.27, p.135-140, 2003.

BURGER, M.E. et al. Acute reserpine and subchronic haloperidol treatments change synaptosomal brain glutamate uptake and elicit orofacial dyskinesia in rats**. Brain Res.**, v.1031, p.202-210, 2005a.

BURGER, M. E. et al. Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain. **Pharmacol. Biochem. Behav.**, v.81, p.608-615, 2005b.

BURGER, M. E. et al. Effects of diphenyl-diselenide on orofcial dyskinesia model in rats**. Brain Res. Bull.**, v.70, p.165-170, 2006.

BUSANELLO, A. et al. Resveratrol protects against a model of vacuous chewing movements induced by reserpine in mice. **Behav. Pharmacol**., v.22, p.71-75, 2011.

BYRNES, J. J.; HAMMER, R. P. The disruptive effect of cocaine on prepulse inhibition is prevented by repeated administration in rats. **Neuropsychopharmacol.**, v.22, p.551-554, 2000.

CADET, J. L.; LOHR, J. B.; JESTE, D. V. Tardive dyskinesia and Schizophrenic burnout: the possible involvement of cytotoxic free radicals. In: Henn FA, DeLisi LE (Eds). Handbook of schizophrenia: **Neurochem. Pharmacol. Schizoph.**, p.425-438, 1987.

CALON, F. et al. Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. **Neuron**, v.43, p.633-645, 2004.

CALON, F. et al. Dietary n-3 polyunsaturated fatty acid depletion activates caspases and decreases NMDA receptors in the brain of a transgenic mouse model of Alzheimer's disease. **European Journal of Neuroscience**, v.22, p.617-626, 2005.

CALON, F.; COLE, G. Neuroprotective action of omega-3 polyunsaturated fatty acids against neurodegenerative diseases: Evidence from animal studies. **Prostaglandins, Leukot. Essent. Fatty Acids**, v.77, p.287-293, 2007.

CALVENTE, P. R. et al. The mitochondrial toxin 3-nitropropionic acid aggravates reserpine-induced oral dyskinesia in rats. **Prog. Neuropsychopharmacol. Biol. Psych.**, v.26, p.401-405, 2002.

CAMARERO, J. et al. Studies, using *in vivo* microdialysis, on the effect of the dopamine uptake inhibitor GBR 12909 on 3,4-methylenedioxymethamphetamine ('ecstasy')-induced dopamine release and free radical formation in the mouse striatum. **J. Neurochem.**, v.81, p.961-972, 2002.

CAMP, D.M.; ROBINSON, T.E. Susceptibility to sensitization. I. Sex differences in the enduring effects of chronic D-amphetamine treatment of locomotion, stereotyped behavior and brain monoamines. **Behav. Brain Res.**, v.30, p. 55-68, 1988

CASEY, D. E. Motor and mental aspects of acute extrapyramidal syndromes. **Acta Psych. Neurol. Scand.**, v.89, p.14-20, 1994.

CHACKO, A.M. et al. Targeted nanocarriers for imaging and therapy of vascular inflammation. **Curr. Opin. Colloid Interface Sci.**, v.16, p. 215-22, 2011.

CHAKOS, M. H. et al. Increase in caudate nuclei volumes of first-episode schizophrenic patients taking antipsychotic drugs. **Am. J. Psych.,** v.151, p.1430-1436, 1994.

CHALON, S. et al. Dietary fish oil induces affects in monoaminergic neurotransmission and behavior in rats. **J. Nutr.**, v.128, p.2512-2519, 1998.

CHEN, W. et al. Development and evaluation of novel itraconazole-loaded intravenous nanoparticles. **Int. J. Pharm.**, v.362, p.133-140, 2008.

CHEN, H. et al. Nanonization strategies for poorly water-soluble drugs. **Drug Discov. Today**, v.16, p.354-360, 2011a.

CHEN, W. et al. Targeted brain delivery of itraconazole via RVG29 anchored nanoparticles. **J. Drug Target.,** v.19, p. 228-234, 2011b.

CHIU, C.C. et al. The effects of omega-3 fatty acids monotherapy in Alzheimer's disease and mild cognitive impairment: A preliminary randomized double-blind placebo-controlled study. **Progr. Neuropsychopharmacol. Biol. Psychiatry**, v.32, p.538-1544, 2008.

CHOUINARD, G. Effects of risperidone in tardive dyskinesia: an analysis of the Canadian multicenter risperidone study. **J. Clin. Psychopharmacol.,** v.15, p.36-44, 1995.

CLIFFORD, J.J. et al. Essential fatty acids given from conception prevent topographies of motor deficits in a transgenic model of Huntington's disease. **Neuroscience**, v.109, p. 81-88, 2002.

COESTER, C. et al. Preparation of avidin-labelled gelatin nanoparticles as carriers for biotinylated peptide nucleic acid (PNA). **Int. J. Pharm.**, v.196, p.147-149, 2000.

COLE, B.J.; KOOB, G.F. Low doses of corticotropin-releasing factor potentiate amphetamine-induced stereotyped behavior. **Psychopharmacology**, v.99, p. 27-33, 1989.

COLE, G.M. et al. Prevention of Alzheimer's disease: Omega-3 fatty acid and phenolic anti-oxidant interventions. **Neurobiol. Aging**, v.26, p.133-136, 2005.

COLPO, G. et al. Ilex paraguariensis has antioxidant potential and attenuates haloperidol-induced orofacial dyskinesia and memory dysfunction in rats. **Neurotox. Res.**, v.12, p.1-10, 2007.

CORTEZ, A.M. et al. Age-dependent effects of K-opioid receptor stimulation on cocaine-induced stereotyped behaviors and dopamine overflow in the caudate-putamen: an in vivo microdialysis study. **Neuroscience**, v.169, p.203-213, 2010.

COUVREUR, P. et al. Poly(cyanoacrylate) nanoparticles as potential lysosomotropic carriers: preparation, morphological and sorptive properties. **J. Pharm. Pharmacol.**, v.31, p.331-332, 1979.

COUVREUR, P.; DUBERNET, C.; PUISIEUX, F. Controlled drug delivery with nanoparticles: current possibilities and future trends. **Eur. J. Pharm. Biopharm.**, v.41, p.2-13, 1995.

COUVREUR, P. et al. Nanocapsule technology: a review. **Crit. Rev. Ther. Drug Carrier Syst.**, v.19, p.99-134, 2002.

COUVREUR, P.; VAUTHIER, C. Nnaotechnology, intelligent design to treat complex disease. **Pharm. Res.**, v.23, p.1417-1450, 2006.

COYLE, J. T.; PUTTFARCKEN, P. Oxidative stress, glutamate, and neurodegenerative disorders. **Science**, v.262, p.689-695, 1993.

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

CREESE I, IVERSEN SD. Blockade of amphetamine induced motor stimulation and stereotype in the adult rat following neonatal treatment with 6-hydroxydopamine. **Brain Res.**, v.55, p.369–382, 1973.

CROW, T. J.; HARRINGTONIS, C. A. Etiopathogenesis and treatment of psychosis. **Ann. Rev. Med.,** v.45, p.219-234, 1994.

CURRAN, C.; BYRAPPA, N.; MCBRIDE, A. Stimulant psychosis: systematic review. **Brit. J. Psych.**, v.185, p.196-204, 2004.

DAMGE, C.; MAINCENT, P.; UBRICH, N. Oral delivery of insulin associated to polymeric nanoparticles in diabetic rats. **J. Control. Release**, v.117, p.163-170, 2007.

DANHIER, F.; FERON, O.; PRÉAT, V. To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. **J. Control. Release**, v.148, p.135-146, 2010.

DARBY, J.K. et al. Haloperidol dose and blood level variability: toxicity and interindividual and intraindividual variability in the nonresponder patient in the clinical practice setting. **J. Clin. Psychopharmcol.**, v.15. p.334-340, 1995.

DATE, A.A.; JOSHI, M.D; PATRAVALE,V.B. Parasitic diseases: liposomes and polymeric nanoparticles versus lipid nanoparticles. **Adv. Drug Deliv. Rev.**, v.59, p.505-521, 2007.

DAVIS, K. L.; KAHN, R. S.; KO, G.; et al. Dopamine in schizophrenia: a review and reconceptualization. **Am. J. Psych.,** v.148, p.1474-1486, 1991.

DAVIS, M. E.; CHEN, Z.; SHIN, D. M. Nanoparticle therapeutics: an emerging treatment modality for cancer. **Nat. Rev. Drug Discovery**, v.*7*, p.771–782, 2008.

DAVSON, H.; SEGAL, M. Physiology of the CSF and Blood–Brain Barrier (CRC Press, Boca Raton), p. 1–192, 1996.

DEEKEN, J.; LOSCHER, W. The blood-brain barrier and cancer: transporters, treatment, and trojan horses. **Clin. Cancer Res.**, v.13, p.1663-1674, 2007.

DELATTRE, A.M. et al. Evaluation of chronic omega-3 fatty acids supplementation on behavioral and neurochemical alterations in 6-hydroxydopamine-lesion model of Parkinson's disease. **Neurosci. Res.**, v.66, p.256-264, 2010.

DENIKER, P. My view of psychopharmacology. In: **Collegium Internationale Neuro-psychopharmacologium** Newsletter: Fall, p. 18-19, 1998.

DHANIKULA, A.B. et al. Long circulating lipid nanocapsules for drug detoxification. **Biomaterials**, v.28, p.1248-1257, 2007.

DICKINSON, S.L.; GADIE, B.; TULLOCH, I.F. α1- and α2-adrenoreceptor antagonists differentially influence locomotor and stereotyped behaviour induced by d-amphetamine and apomorphine in the rat. **Psychopharmacology**, v.96, p.521-527, 1988.

DINGLER, A.; BLUM, R.P.; NIHESUS, H. Solid lipid nanoparticles (SLN/Lipopearls) – a pharmaceutical and cosmetic carrier for the application of vitamin E in dermal products. **J. Microencapsul.**, v.16, p.751-767, 1999.

DOLLERY, C. **Therapeutic drugs**. Edinburgh: Churchill Livingstone, 1991, v.2, p.H1-H4.

DUNN, M.J.; KILLCROSS, S. Clozapine but not haloperidol treatment reverses sub-chronic phencyclidine-induced disruption of conditional discrimination performance. **Behav. Brain Res.**, v.175, p.271–277, 2006.

DURÁN, N.; MATTOSO, L.H.C.; MORAIS, P.C**. Nanotecnologia:** introdução, preparação e caracterização de nanomateriais e exemplos de aplicação. São Paulo: Artliber Editora Ltda, 2006.

EBADI, M.; SRINIVASAN, S. K. Pathogenesis, prevention, and treatment of neuroleptic-induced movement disorders. **Pharmacol. Rev.**, v.47, p.575-604, 1995.

ELKASHEF, A;M.; WYATT, R.J. Tardive dyskinesia: possible involvement of free radicals and treatment with vitamin E. **Schizophr. Bull.**, v. 25, p.731-740, 1999.

EMSLEY, R. et al. Treatment of schizophrenia in low-income countries. International **J. Neuropsychopharmacol.** v.2, p.321-325, 1999.

EMSLEY, R. et al. Randomized, placebo-controlled study of \ethyl-eicosapentaenoic acid as supplemental treatment in schizophrenia. **Am. J. Psychiatry**, v.159, p.1596-1598, 2002.

EMSLEY, D. et al. The effects of eicosapentaenoic acid in tardive dyskinesia: A randomized, placebo-controlled trial. **Schizophr. Res.**, v.84, p.112-120, 2003.

ESTELLE, J.M. et al. Physical characterization of polycaprolactone scaffolds. **J. Mater. Sci. Mater. Med.**, v.19, p.189–195, 2008.

FACHINETTO, R. et al. High fat diet increases the incidence of orofacial dyskinesia and oxidative stress in specific brain regions of rats. **Pharmacol., Biochem. Behav.**, v.81, p.585-592, 2005.

FACHINETTO, R. et al. Valeriana officinalis does not alter the orofacial dyskinesia induced by haloperidol in rats: Role of dopamine transporter**. Prog. Neuropsychopharmacol. Biol. Psych.**, v.31, p.1478-1486, 2007.

FANG, J. et al. Involvment of CYP3A4 and CYP2D6 in the metabolismo of haloperidol. **Cell. Mol. Neurobiol.**, v.17, p.227-223, 1997.

FARAJI, A.H.; WIPF, P. Nanoparticles in cellular drug delivery. **Bioorg. Med. Chem.**, v.17, p.2959-2962, 2009.

FENG, S.S. Nanoparticles of biodegradable polymers for new-concept chemotherapy. **Expert. Rev. Med. Devices**, v.1, 115-125, 2004.

FESSI, H. et al. Nanocapsules formation by interfacial polymer deposition following solvent displacement. **Int. J. Pharm.**, v.113, p.r1-r4, 1989.

FIBIGER, H.; LLOYD, K.G. Neurobiological substrates of tardive dyskinesia: the GABA hypothesis. **Trends Neurosci.**, v.7, p.462-464, 1984.

FILLOUX, F.; TOWNSEND, J.J. Pre- and postsynaptic neurotoxic effects of dopamine demonstrated by intrastriatal injection. **Exp. Neurol.**, v.119, p.79-88, 1993.

FINDLING, R. L. et al. The antipsychotics. **Pediatr. Clin. North Am.,** v.45, p.1205-1233, 1998.

FIRESTONE, D. **Physical and chemical characteristics of oils, fats and waxes**. Champain: AOCS Press,1999.

FLEISCHHACKER, W. W. Tratamento farmacológico da esquizofrenia: uma revisão. In MAJ, M.; SARTORIUS (Orgs.). **Esquizofrenia.** Porto Alegre: Artmed, 2005. p. 71-132.

FLETCHER, P.C.; FRITH, C.D. Perceiving is believing: a Bayesian approach to explaining the positive symptoms of schizophrenia. **Nat. Rev. Neurosci.**, v.10, p.48-58, 2009.

FLORES, F.C. et al. Nanostructured systems containing an essential oil: protection against volatilization. **Quim. Nova**, v.34, p.968-972, 2011.

FONTANA, M.C. et al. Nanoencapsulation as a way to control the release and to increase the photostability of clobetasol propionate: Influence of the nanostructured system. **J. Biomed. Nanotechnol.**, v.5, p.254-263, 2009.

FONTANA, M.C. et al. Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. **Eur. J. Pharm. Biopharm.**, v.79, p. 241-249, 2011.

FORREST, M.L; KWON, F.S. Clinical developments in drug delivery nanotechnology. **Adv. Drug Deliv. Rev.**, v.60, p.861-862, 2008.

FORSMAN, A.; OHMAN, R. Pharmacokinetic studies on haloperidol in man. **Curr. Ther. Res.**, v.20, p.319-336, 1976.

FORSMAN, A. et al. The metabolism of haloperidol in man. **Curr. Ther. Res. Clin. Exp.**, v. 2; p.606–617, 1977.

FRIDOVICH, I. Superoxide dismutases**. Ann. Rev. Biochem.**, v.44, p.147-159, 1975.

FRIEDMAN, A.J. et al. Improved antimicrobial efficacy with nitric oxide releasing nanoparticle generated *S*-nitrosoglutathione. **Nitric Oxide**, v.25, p.381-386, 2011.

FRIESE, A. et al. Increase of the duration of the anticonvulsive activity of a novel NMDA receptor antagonist using poly(butylcyanoacrylate) nanoparticles as a parenteral controlled release system, **Eur. J. Pharm. Biopharm.**, v.49, p.103-109, 2000.

FRONZA, T. et al. **Nanocosméticos:** em direção ao estabelecimento de marcos regulatórios. Porto Alegre: Gráfica da UFRGS, 2007.

FROTA, L.H. Cinqüenta anos de medicamentos antipsicóticos em psiquiatria: I fenotiazinas alifáticas. **J. Bras. Psiq.**, v.50, p.121-41, 2001.

FROZZA, R.L. et al. Characterization of trans-resveratrol-loaded lipid-core nanocapsules and tissue distribution studies in rats. **J. Biomed. Nanotechnol.**, v.6, p.694-703, 2010.

FUENMAYOR, L.D.; DIAZ, S. The effect of fasting on the stereotyped behaviour induced by amphetamine and by apomorphine in the albino rat. **Eur. J. Pharmacol.**, v.99, p.153-158 , 1984.

GABATHULER, R. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. **Neurobiol. Dis.**, v.37, p.48-57, 2010.

GALLEY, H.; DAVIES, M. J.; WEBSTER, N. R. Ascorbil radical formation in patients with sepsis: effects of ascorbate loading. **Free Radic. Biol. Med.**, v. 20, p. 139-143, 1996.

GALILI, R.; MOSBERG, GIL-AD, I. Haloperidol-induced neurotoxicity--possible implications for tardive dyskinesia. **J. Neural. Transm.**, v.107, p.479-490, 2000.

GAO, Y. et al. Prostaglandin E_1 encapsulated into lipid nanoparticles improves its anti-inflammatory effect with low side-effect. **Int. J. Pharm.**, v. 387, p.263-271, 2010.

GARCIA-GARCIA, E. et al. Colloidal carriers and blood–brain barrier (BBB) translocation: A way to deliver drugs to the brain? **Int. J. Pharm.**, v.298, p. 274-292, 2005.

GERLACH, J.; THORSEN, K. The movement pattern of oral tardive dyskinesia in relation to anticholinergic and antidopaminergic treatment. **Int. Pharmacopsychiatry**, v.11, p.1-7, 1976.

GLAZER, W.M. Extrapyramidal side effects, tardive dyskinesia, and the concept of atypicality. **J. Clin. Psych.**, v.61, p.16-21, 2000.

GLICK, I.D.; LEMMENS, P.; VESTER-BLOKLAND, E. Treatment of the symptoms of schizophrenia: a combined analysis of double-blind studies comparing risperidone with haloperidol and other antipsychotic agents. **Int. Clin. Psychopharm.**,v.16, p.265-274, 2001.

GMIRO, V. E.; SERDYUK, S. E. Epinephrine Potentiates Antipsychotic, but not Cataleptogenic Effect of Haloperidol in Rats. **Bull. Exper. Biol. Med. Pharmacol.,** v. 143, n. 5, 2007.

GOLDMAN-RAKIC, P. S. et al. Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction. **Psychopharmacology**, v.174, p.03-16, 2004.

GRANGER, B.; ALBU, S. The haloperidol story. **Ann. Clin. Psychiatry**,. v.17, p.137-140, 2005.

GUPTA, Y.; JAIN, A.; JAIN, S.K. Transferrin-conjugated solid lipid nanoparticles for enhanced delivery of quinine dihydrochloride to the brain. **Journal of Pharmacy and Pharmacology**, v.59, P.935-940, 2007.

GUTERRES, S.S.; ALVES, M.P.; POHLMANN, A.R. Polymeric nanoparticles, nanospheres and nanocapsules for cutaneous applications. **Drug Target Insights**, v.2, p.147-157, 2007.

HAKKARAINEN, M.; ALBERTSSON, A.C. Heterogeneous biodegradation of polycaprolactone — low molecular weight products and surface changes. **Macromol. Chem. Phys.**, v.203, p.1357–1363, 2002.

HAN, Y. et al. Insulin nanoparticle preparation and encapsulation into poly(lactic-co-glycolic acid) microspheres by using an anhydrous system. **Int. J. Pharm.**, v.378, p.159-166, 2009.

HARVEY, R. A.; CHAMPE, P. C. **Farmacologia ilustrada**. 2 ed. Porto Alegre: Artmed, 2002.

HASHIMOTO, M. et al. Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats. **J. Neurochem.**, v.81, p.1084-1091, 2002.

HASHMOTO, M. et al. Docosahexaenoic acid-induced protective effect against impaired learning in amyloid beta-infused rats is associated with increased synaptosomal membrane fluidity. **Clin. Exp. Pharmacol. Physiol.**, v.33, p.934-939, 2006.

HASIRCI, V. et al. Versatility of biodegradable biopolymers: degradability and an in vivo application. **Journal of Biotechnology**, v.86, p.135-150, 2000.

HASSABALLA, H. A.; BALK, R. A.Torsade de pointes associated with the administration of intravenous haloperidol: a review of the literature and practical guidelines for use. **Exp. Opin. Drug Saf.**, v.2, p.543-547, 2003.

HEIMER, L. A new anatomical framework for neuropsychiatric disorders and drug abuse. **Am. J. Psych.,** v.60, p.1726-1739, 2003.

HEALY, D. **The creation of psychopharmacology.** Harvard Univ. Press, Cambridge: MA, 2002.

HENDERSON, D. C. Diabetes mellitus and other metabolic disturbances induced by atypical and antipsychotic. **Curr. Diab. Rep.**, v.2, p.135-140, 2002.

HIA, J.; NASIR, A. Photonanodermatology: the interface of photobiology, dermatology and nanotechnology. **Photoderm. Photoim. Photomed.**, v.27, p.2-9, 2011.

HILLERY, A.M. et al. **Drug delivery and targeting for pharmacists and pharmaceutical scientists**. London: Taylor & Francis, 2001.

HIPPIUS, H. The history of clozapine. **Psychopharmacol.**, v.99, p.S3-S5, 1989.

HOLLEY, F.O. et al. Haloperidol kinetics after oral and intravenous doses. **Clin. Pharmacol. Ther.**, v.33, p.477-484, 1983.

HOWES, O.D.; KAPUR, S. The dopamine hypothesis of schizophrenia: Version III – The final common pathway. **Schizop. Bull**, v.35, p.549-562, 2009.

HUANG, N.Y.; KOSTRZEWA, R.M. Persistent oral dyskinesias in haloperidol-withdrawn neonatal 6-hydroxydopamine-lesioned rats. **Eur. J. Pharmacol.**, v.271, p.433-437, 1994.

HUANG, T.L.; LIOU, C.W.; LIN, T.K. Serum thiobarbituric acid-reactive substances and free thiol levels in schizophrenia patients: Effects of antipsychotic drugs. **Psych. Res.**, v.177, p.18-21, 2010.

IANISKI, F.R. et al. Protective effect of meloxicam-loaded nanocapsules against amyloid-β peptide-induced damage in mice. **Behav. Brain Res.**, v.230, p.100-107, 2012.

IKEDA, H. et al. Effects of chronic haloperidol and clozapine on vacuos chewing and dopamine-mediated jaw movements in rats: evaluation of revised animal model of tardive dyskinesia**. J Neural. Transm.**, v.106, p.1205-1216, 1999.

IMAI, H. et al. Ebselen protects both gray model of focal and white matter in a rodent cerebral ischemia. **Stroke,** v.32, p.2149-2156, 2001.

JANSSEN, P. From haloperidol to risperidone. In: HEALY, D. **The Psychopharmacologists**, London: Arnold, 1998. v.2. p. 39-70.

JAVAID, J.I. Clinical Phamacokinetics of antipsychotics. **J Clin. Pharmacol.**, v.34, p.286-295, 1994.

JAVITT, D.C.; ZUKIN, S.R. Recent advances in the phencyclidine model of schizophrenia. **Am. J. Psych.**, v.148, p.1301-1308, 1991.

JEFFREY, P.; SUMMERFIELD, S. Assessment of the blood-brain barrier in CNS drug discovery. **Neurobiol. Dis.**, v.37, p.33-37, 2010.

JÖNHAGEN, M.E.E. Omega-3 fatty acids in Alzheimer‘s disease. **Alzheimers Dement.**, v.4, p.T199, 2008.

JOY, C.B.; MUMBY-CROFT, R.; JOY, J.A. Polyunsaturated fatty acid supplementation (fish or evening primrose oil) for schizophrenia: A cochrane systematic review. **Schizophr. Res.**, v.41, p.27, 2000.

JUNG, S.H. et al. Amphotericin B-entrapping lipid nanoparticles and their *in vitro* and *in vivo* characteristics. **Eur. J. Pharm. Sci.**, v.37, p.313-320, 2011.

KALGUTKAR, A.S. et al. Assessment of the contributions of cyp3a4 and cyp3a5 in the metabolism of the antipsychotic agent haloperidol to its potentially neurotoxic pyridinium metabolite and effect of antidepressants on the bioactivation pathway. **Am. Society Pharmacol. Exp. Ther.**, v. 31, p. 243-249, 2003.

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

KANE, J. M. et al. Integrating incidence and prevalence of tardive dyskinesia. **Psychopharmacol. Bull.,** v.22, p.254-258, 1986.

KANE, J. et al. Clozapine for the treatmentresistant schizophrenic. A double-blind comparison with chlorpromazine. **Arch. Gen. Psychiatry.**, v.45, p.789– 796, 1988.

KANE, J. M. Dosing issues and depot medication in the maintenance treatment of schizophrenia. **Int. Clin. Psychopharmacol.,** v.10, p.65-71, 1995.

KANEDA, H. et al. Co-administration of progabide inhibits haloperidol-induced oral dyskinesia in rats**. Eur. J. Pharmacol.**, v.212, p.43-49, 1992.

KAPUR, S.; ZIPURSKY, R.; JONES, C. Relationship between dopamine D(2) occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. **Am. J. Psychiatry**, v.157, p.514-20, 2000.

KIM, S.Y.; LEE, Y.M. Taxol-loaded block copolymer nanospheres composed of methoxy poly(ethylene glycol) and poly(epsilon-caprolactone) as novel anticancer drug carriers. **Biomaterials**, v.22, p.1697-1704, 2001.

KIM, D.H.; MARTIN, D.C. Sustained release of dexamethasone form hydrophilic matrices using PLGA nanoparticles for neuronal drug delivery. **Biomaterials**, v.27, p.3031-3037, 2006.

KING, C.; LAKSHMI, N. P.; VORUGANTI, N.P. The evolution of the nomenclature of antipsychotic drugs. **J. Psych. Neurosci.,** v.27, p.168-175, 2002.

KLAWANS, H. L.; RUBOVITS, R. Experimental model of tardive dyskinesia **J. Neur. Transm.**, v.33, p.235-&, 1972.

KNABLE, M. B.; WEINBERGER, D. R. Dopamine, the prefrontal cortex and schizophrenia. **J. Child Adolesc. Psychopharmacol.,** v.11, p.123-131, 1997.

KOO, O.M.; RUBINSTEIN, I.; ONYUKSEL, H. Role of nanotechnology in targeted drug delivery and imaging: a concise review. **Nanomedicine**, v.1, p.193-212, 2005.

KOU, J.; KLORIG, D.C.; BLOOMQUIST, J. R. Potentiating effect of the ATP-sensitive potassium channel blocker glibenclamide on complex I inhibitor neurotoxicity in vitro and in vivo. **Neurotoxicology**, v.27, p.826-834, 2006.

KREUTER, J. Nanoparticulate systems for brain delivery of drugs. **Adv. Drug Deliv. Rev.**, v.47, p.65–81, 2001.

KREUTER, J.; SHAMENKOV, D.; PETROV, V. Apolipoprotein-mediated Transport of Nanoparticle-bound Drugs Across the Blood–Brain Barrier**. J. Drug Targ.**, v.10, p.317-325, 2002.

KREUTER, J. Application of nanoparticles for the delivery of drugs to the brain. **Int. Congr. Ser.**, v.1277, p.85–94, 2005.

KUCZENSKI, R.; SEGAL, D.S. Sensitization of amphetamine-induced stereotyped behaviors during the acute response. **J. Pharmacol. Exp. Ther.**, v.288, p.699-709, 1999.

KUDO, S.; ISHIZAKI, T. Pharmacokinetics of haloperidol: an update.**Clin. Pharmacokinetic.**, v.37, p.435-456, 1999.

KUNKA, R.L.; PEREL, J.M. Haloperidol pharmacokinetics in healthy volunteers. **Curr. Ther. Res.**, v.45, p.1088-1096, 1989.

KUO, W.C; CHUNG, J.F. Physicochemical properties of nevirapine-loaded solid lipid nanoparticles and nanostructured lipid carriers. **Colloids Surf. B: Biointerfaces**, v.83, p. 299-306, 2011.

KUO, Y.C; LIANG, C.T. Inhibition of human brain malignant glioblastoma cells using carmustine-loaded catanionic solid lipid nanoparticles with surface anti-epithelial growth fator receptor. **Biomaterials**, v.32, p.3340-3350, 2011.

KUMAR, M. et al. Intranasal nanoemulsion based brain targeting drug delivery system of risperidone. **Int. J. Pharma.**, v.358, p. 285-291, 2008.

KUMAR, M.; PATHAK, K.; MISRA, A. Formulation and characterization of nanoemulsion-based drug delivery system of risperidone. **Drug Dev. Ind. Pharm.**, v.35, p.387-395, 2009.

LAI, M.L. et al. Effects of fish oil supplementation on acute ischemic brain injury in the rat. **Neurology**, v.43, p.1227-1232, 1993.

LAI, M.K. et al. Application of gold nanoparticles to microencapsulation of thioridazine. **J. Control. Release**, v.111, p.352-361, 2006.

LAMBERT, G. et al. EWS Fli-1 Antisense Nanocapsules Inhibits Ewing Sarcoma-Related Tumor in Mice. **Biochem. Biophys. Res. Com.**, v.279, p.401-406, 2000.

LANGER, R. Polymer-controlled drug delivery systems. **Acc. Chem. Res.**, v.26, p.537–542, 1993.

LAPORTA, M.; ARCHAMBAULT, D.; ROSSCHOUINARD, A. Articulatory impairment associated with tardive-dyskinesia. **J. Nerv. Ment. Dis.,** v.178, p.660-662, 1990.

LARUELLE, M. et al. Increased dopamine transmission in schizophrenia: relationship to illness phases**. Biol. Psych.**, v.46, p.56-72, 1999.

LARUELLE, M. et al. Schizophrenia is associated with increased synaptic dopamine in associative rather than limbic regions of the striatum. **Neuropsychopharmacology**, v.30, p.S196-S196, 2005.

LAVELLE, E.C. et al. The importance of gastrointestinal uptake of particles in the design of oral delivery systems. **Adv. Drug Deliv. Rev.**, v.18, p.5-22, 1995.

LEE, S. et al. Neuroleptic drugs alter the dopamine transporter-mediated uptake and release of dopamine: a possible mechanism for drug-induced tardive dyskinesia. **Pharmacol. Res.,** v.35, p.447-450, 1997.

LEE, K.S. et al., Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast câncer. **Breast Cancer Res. Treat.**, v.108, p.241-250, 2008.

LEWIS, D. A.; LIEBERMAN, J. A. Catching Up on Schizophrenia: Review Natural History and Neurobiology. **Neuron,** v.28, p.325-334, 2000.

LIEBERMAN, J. A.; MAILMAN, R. B.; DUNCAM, G. Serotoninergic basis of antipsychotic drug effects in schizophrenia. **Biol. Psych.,** v.44, p.1099-1117, 1998.

LIN, L.N. et al. Recent advances in nanotechnology based drug delivery to the brain. **Cytotechnology**, v.62, p.377-380, 2010.

LOBENBERG, R. **Smart materials**: applications of nanotechnology in drug delivery and drug targeting. Proceedings of the international conference on MEMS. NANO and Smart Systems (ICMENS'03), 2003.

LOHR, J. B. Oxygen free radicals and neuropsychiatric illness. **Arch. Gen. Psych.,** v.48, p.1097-1106, 1991.

LOHR, J. B.; KUCZENSKI, R.; NICULESCU, A. B. Oxidative mechanisms and tardive dyskinesia. **CNS drugs,** v.17, p.47-62, 2003.

LU, Y.; CHEN, S.C. Micro and nano-fabrication of biodegradable polymers for drug delivery. **Adv. Drug Deliv. Rev.**, v.22, p.1621-1633, 2004.

LYON, M. Animal models of mania and schizophrenia. In: Willner, P. **Behavioural models in psychopharmacology:** theoretical, industrial and clinical perspectives. Cambridge: Cambridge University Press, 1991. p.253-310.

MAGENHEIM, B.; BENITA, S. Nanoparticle characterization a comprehensive physicochemical approach. **S.T.P. Pharma-Sciences**, v.1, p.221-41, 1991.

MAHADIK, P. S.; EVANS, D.; LAL, H. Oxidative stress and role of antioxidant and ω-3 essential fatty acid supplementation in schizophrenia. **Prog. Neuropsychopharmacol. Biol. Psych.,,** v.25, p.463-493, 2001.

MAHAJAN, S.D. et al. Enhancing the delivery of anti retroviral drug "saquinavir" across the blood brain barrier using nanoparticles. **Curr. HIV Res.**, v.8, p.396-404 **,** 2010.

MAINARDES, R.M.; KHALIL, N.M.; GREMIÃO, M.P.D. Intranasal delivery of zidovudine by PLA and PLA–PEG blend nanoparticles. **Int. J. Pharm.**, v.395, p.266-271, 2010.

MANJUNATH, K.; VENKATESWARLU, V. Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration. **J. Control. Release**, v.107, p.215-228, 2005.

MANTZIORIS, F. et al. Biochemical effects of a diet containing foods enriched with n-3 fatty acids. **Am. J. Clin. Nutr.**, v.72, p.42-48, 2000.

MARCHIORI, M.L. et al. Hydrogel containing dexamethasone-loaded nanocapsules for cutaneous administration: preparation, characterization, and in vitro drug release study. **Drug Dev. Ind. Pharm.**, v.36, p.962-971, 2010.

MATSUMURA, Y.; MAEDA, H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agente smancs. **Cancer Res.**, v.46, p.6387–6392, 1986.

MATTHEOLABAKIS, G. et al. In vivo investigation of tolerance and antitumor activity of cisplatin-loaded PLGA-mPEG nanoparticles. **Eur. J. Pharm. Biopharm.**, v.71, p.190-195, 2009.

MAULUDIN, R.; MULLER, R. H.; KECK, C., M. Development of an oral rutin nanocrystal formulation. **Int. J. Pharm.,** v. 370, p. 202–209, 2009a.

MAULUDIN, R.; MULLER, R. H.; KECK, C. M. Kinetic solubility and dissolution velocity of rutin nanocrystals. **Eur. J. Pharm. Sci.**, v. 36, p. 502–510, 2009b.

MAXIA, A. et al. Ethanolic extract of Rubia peregrina L. (Rubiaceae) inhibits haloperidol-induced catalepsy and reserpine-induced orofacial dyskinesia. **Nat. Prod. Res.**, v.26, p.438-445, 2012.

MELO, N.F.S. et al. Desenvolvimento e Caracterização de Nanocápsulas de Poli (L-Lactídeo) Contendo Benzocaíne. **Quim. Nova**, v.33, p.65-69, 2010.

MENEGATTI, R. et al. Esquizofrenia: quarenta anos da hipótese dopaminérgica sob a ótica da Química Medicinal. **Quím. Nova,** v.27, p.447-455, 2004.

MESHUL, C. K.; TAN, S. E. Haloperidol-induced morphological alterations associated with changes in calcium/calmodulin kinase II activity and the glutamate immunoreactivity. **Synapse**, v.18, p.205-217**,** 1994.

McCORD, J.M.; FRIDOVICH, I. Superoxide dismutase an enzymic function fpr erythrocuprein (hemocuprein). **J. Biol. Chem.**, v.244, p.6049-6055, 1969.

MIGUEL, L., Formulações com óleo de sementes de uva "Suvalan". Porto Alegre: Faculdade de Farmácia da UFRS, 1983.

MILJEVIĆ, C. et al. Correlation analysis confirms differences in antioxidant defence in the blood of types I and II schizophrenic male patients treated with anti-psychotic medication. **Psych. Res.,** v.178, p.68-72, 2010.

MILLAN, M.J.; GRESSIER, H., BROCCO, M. The dopamine D_3 receptor antagonist, (+)-S 14297, blocks the cataleptic properties of haloperidol in rats**. Eur. J. Pharmacol.**, v.321, p.R7-R9, 1997.

MILLER, R.; CHOUINARD, G. Loss of striatal cholinergic neurons as a basis for tardive and L-dopa-induced dyskinesias, neuroleptic-induced supersensitivity psychosis and refractory schizophrenia. **Biol. Psych.,** v.34, p.713-738, 1993.

MIMCHE, P.N.; TARAMELLI, D.; VIVAS, L. The plant-based immunomodulator curcumin as a potential candidate for the development of an adjunctive therapy for cerebral malaria. **Malar. J.**, v.10, art. no. S10, 2011.

MION, C. C. et al. MRI abnormalities in tardive dyskinesia. **Psych. Res.**, v.40, p.157-166, 1991.

MITTAL, G. et al. Development and evaluation of polymer nanoparticles for oral delivery of estradiol to rat brain in a model of Alzheimer's pathology. **J. Control. Release**, v.150, p.220-228, 2011.

MOGHIMI, S.M.; HUNTER, A.C.; MURRAY, J.C. Nanomedicine: current status and future prospects. **FASEB J.**, v.19, p.311-330, 2005.

MOHAMMADI, G. et al. Development of azithromycin–PLGA nanoparticles: Physicochemical characterization and antibacterial effect against Salmonella typhi. **Colloids Surf. B Biointerfaces**, v.80, p.34-39, 2010.

MOOR, M.J. et al. Adipose Tissue Distribution and Chemical Structure of Basic Lipophilic Drugs: Desipramine, N-Acetyl Desipramine, and Haloperidol. **Pharmacol. Toxicol.**, v.70, p.121–124, 1992.

MOORE, N.A. et al. The behavioral pharmacology of olanzapine, a novel atypical antipsychotic agent. **J. Pharmacol. Exp. Ther**., v.262, p.545-551, 1992.

MOORE, S.; KENYON, P. Atypical antipsychotics, clozapine and sulpiride do not antagonise amphetamine-induced stereotyped locomotion. **Psychopharmacology**, v.114, p.123-130, 1994.

MORENO, R.A. et al. Anticonvulsivantes e antipsicóticos no tratamento do transtorno bipolar. **Rev. Bras. Psiquiatr**., v.26, p.37-43, 2004.

MORGAN, M.G. et al. Prospective analysis of premature mortality in schizophrenia in relation to health service engagement: a 7.5 year study within an epidemiologically complete, homog-enous population in rural Ireland. **Psychiat Res**, v.117, p.127–35, 2003.

MORRIS, R. Developments of a water-maze procedure for studying spatial learning in the rat. **J. Neurosci. Methods,** v.11,p.47–60, 1984.

MOTAMAN, S.; BARYAR, F.; FARZIN, A. Effects of beta-carbolines harmane, norharmane and harmine on apomorphine-induced stereotyped behaviors in rat. **Behav. Pharmacol.**, v.16, p.S105-S105, 2005.

MU, L.; FENG, S.S. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol): PLGA nanoparticles containing vitamin E TPGS. **J. Control. Release**, v.86, p.33-48, 2003.

MU, L.; SPRANDO, R.L. Application of nanotechnology in cosmetics. **Pharm. Res.**, v.27, p.1746–1749, 2010.

MULLER, R.H. et al. In vitro characterization of poly (methyl-methacrylate) nanoparticles and correlation to their invivo fate. **J. Control. Release**, v.20, p.237-246, 1992.

MÜLLER, R.H.; MÄDER, K.; GOHLA, S. Solid lipid nanoparticles (SLN) for controlled drug delivery - review of the state of the art. **Eur. J. Pharm. Biopharm.**, v.50, p.161-177, 2000.

MURSEL, K. et al. The antioxidant effect of wheat germ oil on subchronic coumaphos exposure in mice. **Ecotoxicol. Environ. Saf.**, v.74, p.2119-2125 , 2011.

MURTHY, R.S.R. Biodegradable polymers. In: JAIN, N.K. (Eds.), Controlled and novel drug delivery. CBS Publisher, New Delhi, 1997. p. 27–51,

MUTHU, M.S.; SINGH, S. Studies on biodegradable polymeric nanoparticles of risperidone: in vitro and in vivo evaluation. **Nanomedicine**, v. 3, p. 305-319, 2008.

MUTHU, M.S.; SINGH, S. Targeted nanomedicines: effective treatment modalities for cancer, AIDS and brain disorders**. Nanomedicine,** v. 4, p. 105-118, 2009.

MUTHU, M.S. et al. PLGA nanoparticle formulations of risperidone: preparation and neuropharmacological evaluation. **Nanomedicine**, v.5, p. 323-333, 2009.

NAGARWAL, R. C. et al. Polymeric nanoparticulate system: a potential approach for ocular drug delivery. **J. Control. Release**, v.136, p.2-13, 2009.

NAIDU, P.S.; KULKARNI, S.K. Possible involvement of prostaglandins in haloperidol-induced orofacial dyskinesia in rats. **Eur. J. Pharmacol**., v.430, p.295-298, 2001.

NAIDU, P.S., SINGH, A., KULKARNI, S.K. Quercetin, a bioflavonoid, attenuates haloperidol-induced orofacial dyskinesia. **Neuropharmacol.**, v.44, p.1100-1106, 2003.

NATIONAL SCIENCE FOUNDATION (NSF). **Nanotechnology definition.** Virginia: USA, 2000.

NIEMEGEERS, C.J.E. Pharmacology and mechanism of action of neuroleptics haloperidol and haloperidol decanoate. Workshop haloperidol decanoate. Departament of Pharmacology, Janssen Pharmaceutics Belgium:01-13, 1983.

NORMAN, M.E.; WILLIAMS, P.; ILLUM, L. Human serum-albumin as a probe for surface conditioning (opsonization) of block copolymer-coated microspheres. **Biomaterials**, v.13, p.841–849, 1992.

OHKAWA, H.; OHISHI, N.; YAGI, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Anal. Biochem.**, v.95, p.351-358, 1979.

OLIVEIRA, S. S. A. M. **Preparação e caracterização in vitro de micropartículas de heparina fracionada potencialmente aplicáveis ao tratamento da trombose venosa profunda.** Dissertação (Mestrado em Ciências Farmacêutica) Universidade de São Paulo, Ribeirão Preto, 2009.

OMS. **The world health report 2000:** health system: improving performance. Genebra: OMS, 2000.

OURIQUE, A. F. et al. Lipid-core nanocapsules as a nanomedicine for parenteral administration of tretinoin: development and in vitro antitumor activity on human myeloid leukaemia cells. **J. Biomed. Nanotechnol.**, v.6, p.214-223, 2010.

OURIQUE, A.F. et al. Improved photostability and reduced skin permeation of tretinoin: Development of a semisolid nanomedicine. **Eur. J. Pharm. Biopharm.**, v.79, p.95-101, 2011.

OWENS, C.W.I.; BELCHER, J. A colorimetric micro-method for the determination of glutathione**. Biochem. J.**, v.94, p.705-711, 1965.

PANDEY, R.; KHULLER, G.K. Oral nanoparticle-based antituberculosis drug delivery to the brain in an experimental model. **J. Antimicrob. Chemother.**, v.57, p.1146-1152, 2006.

PARDEIKE, J. et al. Development of an Itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application. **Int. J. Pharm.**, v.419, p.329-338, 2011.

PARIKH, V.; KHAN, M.M.; MAHADIK, S.P. Differential effects of antipsychotics on expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. **J. Psychiatr. Res.**, v.37, p.43-51, 2003.

PARIHK, T.; BOMMANA, M.M.; SQUILLANTE, E. Efficacy of surface charge in targeting pegylated nanoparticles of sulpiride to the brain **. Eur. J. Pharm. Biopharm.**, v.74, p.442-450, 2010.

PARK, K. Nanotechnology: What it can do for drug delivery. **J. Control. Release**, v.120, p.1-3, 2007.

PASSOS, C.P. et al. Enhancement of grape seed oil extraction using a cell wall degrading enzyme cocktail. **Food Chem.**, v.115, p.48-53, 2009.

PERACCHIA, M.T. Stealth nanoparticles for intravenous administration. **S.T.P. Pharma Sci.**, v.13, p.155–161, 2003.

PONTO, T. et al. A prospective study on the pattern of medication use for schizophrenia in the outpatient pharmacy department, Hospital Tengku Ampuan Rahimah, Selangor, Malaysia. **Methods Find. Exp. Clin. Pharmacol.**, v.32, p.427-32, 2010.

PRIPREM, A. et al. Anxiety and cognitive effects of quercetin liposomes in rats. **Nanomedicine: Nanotechnology, Biology and Medicine**, v.4, p.70-78, 2008.

PROESQ. **Programa de esquizofrenia UNIFESP-EPM.** São Paulo, 2007.

RAJA, M. Tardive dystonia: prevalence, risk factors and comparison with tardive dyskinesia in a population of two hundred acute psychiatric in patients. **Eur. Arch. Psych. Clin. Neurosc.,** v.245, p.145-151, 1995.

RANG, H.P. et al. Farmacologia. In:_____. **Substâncias antipsicóticas.** 5. ed. Elsevier: Rio de Janeiro, 2004.

RASMUSSEN, K. et al. The orexin-1 antagonist SB-334867 blocks antipsychotic treatment-emergent catalepsy: Implications for the treatment of extrapyramidal symptoms. **Schizoph. Bull.**, v.33, p.1291-1297, 2007.

REYNOLDS, G. P. Receptor mechanisms in the treatment of schizophrenia. **J. Clin. Psychopharmacol.,** v.18, p.340-345, 2004

REZA, F. et al. Antioxidant activity of sesame, rice bran and bene hull oils and their unsaponifiable matters. **Eur. J. Lipid Sci. Technol.**, v.113, p.506-512, 2011.

RIGO, L.A. **Desenvolvimento de plataforma tecnológica a partir de sistemas nanoparticulados obtidos por óleos vegetais cultivados na região central do Estado do Rio Grande do Sul.** Dissertação (Mestrado em Ciências Farmacêuticas) Universidade Federal de Santa Maria, Santa Maria, 2011.

ROBINSON, T. E.; BECKER, J. B. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis, **Brain Res. Rev.**, v.11, p. 157–198, 1986.

ROCHA, J.B.T. et al. Undernutrition during suckling changes the sensitivity to haloperidol and chlorpromazine in two behavioral measures in weaning Rats**. Pharmacol. Toxicol.**, v.81,p.114-123, 1997.

ROFFMAN, J.L.; RASKIN, L.A. Stereotyped behavior: effects of d-amphetamine and methylphenidate in the young rat. **Pharmacol. Biochem. Behav.**, v.58, p.1095-1102, 1997.

ROSENHECK, R. et al. Impact of clozapine on negative symptoms and on the deficit syndrome in refractory schizophrenia. **Am. J. Psychiatry**, v.156, p.88-93, 1999.

RUKMINI, M.S.; D'SOUZA, B.; D'SOUZA, V. Superoxide dismutase and catalase activities and their correlation with malondialdehyde in schizophrenic patients. **Indian J. Clin. Biochem.**, v.19. p.114-118, 2004.

SACHDEV, P. S. The current status of tardive dyskinesia. **Aust. New Zeal. J. Psych.,** v.34, p.355-369, 2000.

SAHOO, S.K.; LABHASETWAR, V. Nanotech approaches to drug delivery and imaging. **Drug Discov. Today**, v.8, p.1112-1123, 2003

SAKATA, S. et al. Programming control of intelligent drug releases in response to single and binary environmental stimulation signals using sensor and electroresponsive hydrogel.**Radiat. Phys. Chem.**, v.76, p.733-737, 2007.

SALGUEIRO, A. et al. An inductively coupled plasma method for determination of cyclophosphamide loaded to polymeric systems. **J. Pharmac. Biomed. Anal.,** v.21, p.611-618, 1999.

SAMADI, P. et al. Docosahexaenoic acid reduces levodopa-induced dyskinesias in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine monkeys. **Ann. Neurol.**, v.59, p.282-288, 2006.

SAMS-DODD, F. Effects of diazepam, citalopram, methadone and naloxone on PCP-induced stereotyped behaviour and social isolation in the rat social interaction test. **Neurosci. Biobeh. Rev.**, v.23, p.287-293, 1998a.

SAMS-DODD, F. Effects of continuous D-amphetamine and phencyclidine administration on social behaviour, stereotyped behaviour, and locomotor activity in rats. **Neuropsychopharmacology**, v.19, p.18-25, 1998b.

SANTOS, R.M.M.; FIALHO, S.L. Nanopartículas. **Biotecnologia Ciência e Desenvolvimento**, Ano 10, n 37, jan./fev. 2007.

SARANDOL, A. et al. Oxidative–antioxidative systems and their relation with serum S100 B levels in patients with schizophrenia: Effects of short term antipsychotic treatment. **Prog. Neuropsychopharmacol Biol. Psychiatry,** v.31, p.1164-1169, 2007.

SARSILMAZ, M. et al. Potential role of dietary ϖ-3 essential fatty acids on some axidant/antioxidant parameters in rats' corpus striatum. **Prostaglandins, Leukot. Essent. Fatty Acids**, v.69, p.253-259, 2003a.

SARSILMAZ, M. et al. Potential role of dietary omega-3 essential fatty acids on oxidant/antioxidant balance in rat hippocampus. **Neurosci. Res. Commun.**, v.33, p.114-123, 2003b.

SAWA, A.; SNYDER, S.W. Schizophrenia: diverse approaches to a complex disease. **Science**, v.296, p.692-695, 2002.

SCHAFFAZICK, S.R. et al. Caracterização e estabilidade físico-química de sistemas poliméricos nanoparticulados para administração de fármacos. **Quím. Nova**, v.26, p.726-737, 2003.

SCHAFFAZICK, S.R. et al. Incorporation in polymeric nanocapsules improves the antioxidante effect of melatonin against lipid peroxidation in mice brain and liver. **Eur. J. Pharm. Biopharm.**, v.69, p.64–71, 2008.

SCHRÖDER, N. et al. Impairing effects of chronic haloperidol and clozapine treatment on recognition memory: possible relation to oxidative stress. **Schizophr. Res.**, v.73, p.377-378, 2005.

SEE, R. E.; CHAPMAN, M. A. Chronic haloperidol, but not clozapine, produces altered oral movements and increased extracellular glutamate in rats. **Eur. J. Pharmacol.**, v.263, p.269-276, 1994.

SEEMAN, P. et al. Brain receptors for antipsychotic drugs and dopamine: direct binding assays. **Proc. Natl. Acad. Sci. U. S. A.**, v.72, p.4376-4380, 1975.

SEEMAN, P. et al. Antipsychotic drug doses and neuroleptic/dopamine receptors. **Nature**, v.261, p.717-719, 1976.

SEJU, U.; KUMAR, A.; SAWANT, K.K. Development and evaluation of olanzapine-loaded PLGA nanoparticles for nose-to-brain delivery: In vitro and in vivo studies. **Acta Biomater.**, article in press, doi:10.1016/j.actbio.2011.07.025, 2011.

SHAFIEE, M. et al. Grape seed extract capacities at protecting LDL against oxidation generated by Cu^{2+}, AAPH or SIN-1 and at decreasing superoxide THP-1 cell production. A comparison to other extract or compounds. **Free Radic. Res.**, v.37, p.573-584, 2003.

SHARM, S. et al. Pro-Leu-glycinamide and its peptidomimetic, PAOPA, attenuate haloperidol induced vacuous chewing movements in rat: A model of human tardive dyskinesia. **Peptides**, v.24, p.313-319, 2003.

SHI, J. et al. Polyphenolics in grape seeds-biochemistry and functionality**. J. Med. Food**, v.6, p 291–299, 2003.

SHIIGI, Y.; CASEY, D.E. Behavioral effects of ketamine, an NMDA glutamatergic antagonist, in non-human primates. **Psychopharmacology**, v.146, p.67–72, 1999.

SHIRAKAWA, I.; BRESSAN, R.A.; CHAVES, A.C. Tratamento farmacologico da esquizofrenia. In: SHIRAKAWA, I.; CHAVES, A.C.; MARI, J.J. **O desafio da esquizofrenia**. Sao Paulo: Lemos Editorial, 2001. p.135-148.

SIEGEL, S. J.; WINEY, K. I.; GUR, R. E. Surgically implantable long-term antipsychotic delivery systems for the treatment of schizophrenia. **Neuropsychopharmacol.,** v.26, p.817-823, 2002.

SILVA, Penildon. **Farmacologia.** 6. ed. Rio de Janeiro: Guanabara Koogan, 2002.

SILVA, R.C.B. Esquizofrenia: uma revisão. **Psicologia USP**, v.17, p.263-285, 2006.

SILVA, A.C. et al. Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): high pressure homogenization versus ultrasound. **Colloids Surf. B: Biointerfaces**, v.86, p.158–165, 2011.

SILVEIRA, I.D. **O efeito do uso crônico de haloperidol associado à dieta com alto teor de lipídio na peroxidação lipídica no fígado de ratos wistar.** Dissertação (Mestrado em Bioquímica). Universidade Federa de Santa Maria, Santa Maria, 2007.

SILVERS, K.M.; WOOLLEY, C.C; HAMILTON, F.C. Randomised double-blind placebo-controlled trial of fish oil in the treatment of depression. **Prostaglandins, Leukot. Essent. Fatty Acids**, v.72, p.211-218, 2005.

SINCLAIR, A.J. Omega 3 in depression: from epidemiology to treatment **Asian J. Psychiatr.**, v.4, p.S9, 2011.

SINGH, S.; MUTHU, M.S. Preparation and characterization of nanoparticles containing an atypical antipsychotic agent**. Nanomedicine,** v.2, p.233-240 , 2007.

SINHA, V.R. et al. Poly-epsilon-caprolactone microspheres and nanospheres: an overview. **Int. J. Pharm.**, v.278, p.1–23, 2004.

SINHA, R.A. et al. Anti-apoptotic role of omega-3-fatty acids in developing brain: perinatal hypothyroid rat cerebellum as apoptotic model. **Int. J. Dev. Neurosci.**, v.27, p.377-383, 2009.

SIVRIOGLU, E.Y. et al. The impact of ω-3 fatty acids, vitamins E and C supplementation on treatment outcome and side effects in schizophrenia patients treated with haloperidol: An open-label pilot study . **Prog. Neuropsychopharmacol. Biol. Psychiatry**, v.31, p.1493-1499, 2007.

SNYDER, S.H. The dopamine hypothesis of schizophrenia focus on dopamine receptors. **Am. J. Psychiatry**, v.133, p.192-202, 1976.

SNYDER, S. H. **Drugs and the brain.** New York: Scientific American Library, 1986.

SONGUR, A. et al. Hypothalamic superoxide dismutase, xanthine oxidase, nitric oxide and malondialdehyde in rats fed with fish ω-3 fatty acids**. Prog. Neuropsychopharmacolo. Biol. Psychiatry**, v.28, p.693-698, 2004.

SOPPIMATH, K.S. et al. Biodegradable polymeric nanoparticles as drug delivery devices. **J. Control. Release**, v.70, p.1-20, 2001.

STANSBY, M.E. Nutritional properties of fish oils. **World Rev. Nutr. Diet.**, v.11, p.46-105, 1969.

SURI, S.S.; FENNIRI, H.; SINGH, B. Nanotechnology-based drug delivery systems. **J. Occup. Med. Toxicol**, v.2, p.16-22, 2007.

TAO, S.L.; DESAI, T.A. Microfabricated drug delivery systems from particles to pores. **Adv. Drug Del. Rev.**, v.55, p.315-328, 2003.

TADA, M. et al. Combined treatment of quetiapine with haloperidol in animal models of antipsychotic effect and extrapyramidal side: comparison with risperidone and chlorpromazine. **Psychopharmacol.**, v.176, p.94-100, 2004.

TEIXEIRA, A.M. et al. Beneficial effects of an innovative exercise model on motor and oxidative disorders induced by haloperidol in rats. **Neuropharmacology**, v.60, p.432-438, 2011.

TEIXEIRA, A.M. et al. Could dietary trans fatty acids induce movement disorders? Effects of exercise and its influence on Na+K+-ATPase and catalase activity in rat striatum. **Behav. Brain Res.**, v.226, p.504– 510, 2012.

THEME-FILHA, M.M.; SZWARCWALD, C.L.; SOUZA-JÚNIOR, P.R.B. Socio-demographic characteristics, treatment coverage, and self-rated health of individuals who reported six chronic diseases in Brazil. **Cad. Saúde Pública**, v.21, p.S43-S53, 2005.

TIRONE, F.; PARENTI, M.; GROPPETTI A. Opiate and dopamine stimulate different GTPase in striatum: evidence for distinct modulatory mechanisms of adenylate cyclase. **J. Cyclic Nucleot. Prot. Phosph. Res.**, v.10, p.327-339, 1985.

TREVIZOL, F. et al. Comparative study between two animal models of extrapyramidal movement disorders: Prevention and reversion by pecan nut shell aqueous extract. **Behav. Brain Res.,** v.221, p.13–18, 2011.

TSAI, G. et al. Markers of glutamatergic neurotransmission and oxidative stress associated with tardive dyskinesia. **Am. J. Psychiatry**, v.155, p.1207–13, 1998.

UJIKE, H., AKIYAMA, K., OTSUK, S. D2 but not D1 dopamine agonists produce augmented behavioral response in rats after subchronic treatment with methamphetamine or cocaine. **Psychopharmacol**, v.102, p.459–464, 1990.

UKAI, W. et al. Neurotoxic potential of haloperidol in comparison with risperidone. Implication of Aktmediated signal changes by haloperidol. **J. Neural Transm.**, v.111, p.667-681, 2004.

VADDADI, K.S. et al. A double-blind trial of essential fatty acid supplementation in patients with tardive dyskinesia. **Psychiatry Res.**, v.27, p.313-323, 1989.

VERMA, R.K.; GARG, S. Current status of drug delivery technologies and future directions. **Pharmac. Technol.**, v.25, p.1-4, 2001.

VENKATESWARLU, V.; MANJUNATH, K. Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. **J. Control. Release**, v.95, p.627-638, 2004 .

VERCAUTEREN, D. et al. On the cellular processing of non-viral nanomedicines for nucleic acid delivery: Mechanisms and methods. **J. Control. Release**, v. 5, n. 20, 2012.

VISIOLI, F.; POLI, A.; GALL, C. Antioxidant and other biological activities of phenols from olives and olive oil. **Med. Res. Rev.**, v.22, p.65-75, 2002.

VIVEK, K.; REDDY, H.; MURTHY, R.S.R. Investigations of the effect of the lipid matrix on drug entrapment, in vitro release, and physical stability of olanzapine-loaded solid lipid nanoparticles. **Pharm. Sci. Tech.**, v.8, p.16-24, 2007.

VÕIKAR, V. et al. Apomorphine-induced behavioural sensitization in rats: individual differences, role of dopamine and NMDA receptors. **Eur. Neuropsychopharmacol.**, v.9, p.507–514, 1999.

VRIGNAUD, S.; BENOIT, J.P.; SAULNIER, P. Strategies for the nanoencapsulation of hydrophilic molecules in polymer-based nanoparticles. **Biomaterials**, v.32, p.8593-8604, 2011.

XU, N. et al. Efficacy of amphotericin B-polybutylcyanoacrylate nanoparticles against cryptococcal meningitis in mice. **Chinese J. Biomed. Eng.**, v.28, p.285-288, 2009.

XU, K. et al. Efficacy of CG3R6TAT Nanoparticles Self-Assembled from a Novel Antimicrobial Peptide for the Treatment of Candida albicans Meningitis in Rabbits. **Chemotherapy**, v.57, p.417-425, 2011.

YAMAGUCHI, F. et al. Free radical scavenging activity of grape seed extract and antioxidants by electron spin resonance spectrometry in an H_2O/NaOH/DMSO system. **J. Agric. Food Chem.**, v.47, p.2544-2548, 1999.

YAN, L.-Y.; TRABER, M.G.; PACKER, L. Spectrophotometric method for determination of carbonyls in oxydatively modified apolipoprotein B of human low-density lipoproteins. **Anal.l Biochem.**, v.228, p.349-351, 1995.

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

YEN, Y.C.; LUNG, F.W.; CHONG, M.Y. Adverse effects of risperidone and haloperidol treatment in schizophrenia. **Prog. Neuropsychopharmacol. Biol. Psych.**, v.28, p.285-290, 2004.

YEN, F.L. et al. Nanoparticles formulation of Cuscuta chinensis prevents acetaminophen-induced hepatotoxicity in rats. **Food Chem. Toxicol.**, v.46, p.1771-1777, 2008.

YEHUDA, S.; RABINOVITZ, S.; MOSTOFSKY, D.I. Essential fatty acids and the brain: From infancy to aging. **Neurobiol. Aging**, v.26, p.98-102, 2005.

YOKOYAMA, M.; OKANO, T. Targetable drug carriers: present status and a future perspective. **Adv. Drug Deliv. Rev.**, p.77-80, 1996.

WAGNER, V. et al. The emerging nanomedicine landscape. **Nat. Biotechnol.**, v.24, p.1211–1217, 2006.

WAINWRIGHT, P.E. Dietary essential fatty acids and brain function: a development perspective on mechanisms. **Proc. Nutr. Soc.**, v.61, p.61-69, 2002.

WANG, C. et al. n–3 Fatty acids from fish or fish-oil supplements, but not α-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. **Am. J. Clin. Nutr.**, v. 84, p.5-17, 2006.

WANG, H. et al. The efficacy of self-assembled cationic antimicrobial peptide nanoparticles against Cryptococcus neoformans for the treatment of meningitis. **Biomaterials**, v.31, p.2874-2881 , 2010a.

WANG, H. et al. The efficacy of self-assembled cationic antimicrobial peptide nanoparticles against Cryptococcus neoformans for the treatment of meningitis. **Biomaterials** v.31, p.2874-2881, 2010b.

WEN, Z. et al. Odorranalectin-conjugated nanoparticles: Preparation, brain delivery and pharmacodynamic study on Parkinson's disease following intranasal administration **J. Control. Release**, v.151, p.131-138, 2011.

WOERNER, M.G.; ALVIR, J.M.J.; SALTZ, B.L. Prospective study of tardive dyskinesia in the elderly: Rates and risk factors. **Am. J. Psych.**, v.155, p.1521-1528, 1998.

WOHLFART, S. et al. Kinetics of transport of doxorubicin bound to nanoparticles across the blood–brain barrier. **J. Control. Release**, v.154, p.103-107, 2011.

WOLGIN D.L.; JAKUBOW, J.J. Tolerance to amphetamine hypophagia: A real-time depiction of learning to suppress stereotyped movements in the rat. **Behav. Neurosci.,** v.118, p.470-478, 2004.

WONG, H.L. et al. Nanotechnology applications for improved delivery of antiretroviral drugs to the brain. **Adv. Drug Deliv. Rev.,** v.62, p.503-517, 2010.

WONG, H.L.; WU, X.Y.; BENDAYAN, R. Nanotechnological advances for the delivery of CNS therapeutics. **Adv. Drug Deliv. Rev.**, v.64, p.686-700,2012.

WOODRUFF, M.A.; HUTMACHER, D.W. The return of a forgotten polymer–polycaprolactone in the 21st century. **Prog. Polym. Sci.**, v.35, p.1217-1256, 2010.

WU, Q. et al. Concurrent autoreceptor-mediated control of dopamine release and uptake during neurotransmission: an in vivo voltammetric study. **J. Neurosci.**, v.22, p.6272-6281, 2002.

WU, A.; YING, Z.; GOMEZ-PINILLA, F. Dietary omega-3 fatty acids normalize BDNF levels, reduce oxidative damage, and counteract learning disability after traumatic brain injury in rats. **J. Neurotrauma**, v.21, p.1457-1467, 2004.

WU, T.H. et al. Preparation, physicochemical characterization, and antioxidant effects of quercetin nanoparticles. **Int. J. Pharm.**, v.346, p.160–168, 2008.

ZARARSIZ, I. et al. Protective effects of omega-3 essential fatty acids against formaldehydeinduced neuronal damage in prefrontal cortex of rats. **Cell Biochem. Funct.**, v.24, p.237–244, 2006.

ZHU, B.T. CNS dopamine oxidation and catechol-O-methyltransferase: Importance in the etiology, pharmacotherapy, and dietary prevention of Parkinson's disease. **Int. J. Mol. Med.**, v.13, p.343-353, 2004.

ZHU, L. et al. Chitosan-coated magnetic nanoparticles as carriers of 5-Fluorouracil: Preparation, characterization and cytotoxicity studies. **Colloids Surf. B: Biointerfaces**, v.68, p.1-6, 2009.

ZHURAVLIOVA, E.; BARBAKADZE, T.; NATSVLISHVILI, N. Haloperidol induces neurotoxicity by the NMDA receptor downstream signaling pathway, alternative from glutamate excitotoxicity. **Neurochem. Int.**, v.50, p.976-982, 2007.