UNIVERSIDADE FEDERAL DO PARANÁ

RAISA WENDHAUSEN GRADOWSKI

# EFEITO TIPO-ANTIDEPRESSIVO DA CURCUMINA NO MODELO ANIMAL DA 6-OHDA DE DOENÇA DE PARKINSON

CURITIBA

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# EFEITO TIPO-ANTIDEPRESSIVO DA CURCUMINA NO MODELO ANIMAL DA 6-OHDA DE DOENÇA DE PARKINSON

Dissertação apresentada como requisito parcial para a obtenção do título de Mestre em Farmacologia, Curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientadora: Profª. Dra. Maria Aparecida B.F.Vital

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Programa de Pós-Graduação em Farmacologia

# PARECER

A Comissão Examinadora da Dissertação de Mestrado "Efeito tipo-antidepressivo da curcumina no modelo animal da 6-OHDA de doença de Parkinson", de autoria da pós-graduanda **RAISA WENDHAUSEN GRADOWSKI**, sob orientação da Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital e composta pelos professores: Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Presidente - Farmacologia - UFPR); Prof.ª Dr.ª Lia Sumie Nakao (Patologia Básica – UFPR) e Prof. Dr. Marcelo Meira Santos Lima (Fisiologia - UFPR), reuniu-se e, de acordo com o Regimento Interno do Programa de Pós-Graduação em Farmacologia, a pós-graduanda foi APROVADA. Para a devida publicação o trabalho deverá sofrer as modificações sugeridas, que serão conferidas por sua orientadora. Em Curitiba, 28 de junho de 2013.

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*“A mente que se abre a uma nova ideia jamais voltará ao seu tamanho original.”*

**Albert Einstein**

## RESUMO

Um dos sintomas não motores mais comuns na Doença de Parkinson (DP) é a depressão, que afeta aproximadamente 35% dos pacientes e pode levar a uma redução significativa da qualidade de vida dos mesmos. A curcumina, o principal composto ativo da *Curcuma longa* (tumeric), vem apresentando diversas propriedades farmacológicas como atividade anti-inflamatória, inibidora da monoaminooxidase (MAO) e efeitos neuroprotetores. Este composto já tem sido extensivamente estudado em vários modelos de depressão e, mais recentemente, em modelos de DP. Mas nada é sabido sobre seus efeitos na depressão associada à DP. Com isso, o presente estudo investigou o efeito tipo-antidepressivo da curcumina no modelo animal da 6-hidroxidopamina (6-OHDA) de DP. Ratos machos Wistar foram aleatoriamente distribuídos em 4 grupos (n=9-14/grupo): sham-veículo; sham-curcumina; 6-OHDA-veículo e 6-OHDA-curcumina. Os animais dos grupos 6-OHDA receberam esta neurotoxina for infusão intranigral, enquanto os dos grupos sham receberam veículo. Todos os ratos foram tratados por 21 dias com curcumina (30mg/kg, v.o.) ou veículo (óleo de girassol, v.o.), iniciando-se 1 hora após a cirurgia. 24 horas e 21 dias após a cirurgia os animais foram submetidos ao teste do campo aberto. Imediatamente depois, os ratos foram avaliados no teste de natação forçada modificado (TNFm). Para o teste de preferência pela sacarose, os procedimentos foram realizados primeiramente antes da cirurgia e depois semanalmente se iniciando 7 dias depois da cirurgia, até o 21° dia. A quantificação dos neurônios da substância negra *pars compacta* (SNpc) tirosina hidroxilase (TH)-positivos e das monoaminas no estriado e hipocampo foi realizada 21 dias após a cirurgia. Os animais dos grupos 6-OHDA apresentaram uma redução nos parâmetros locomotores 1 dia após a cirurgia, que foram revertidos no 21°dia. Os animais 6-OHDA tratados com curcumina mostraram um efeito tipo-antidepressivo tanto no TNFm como no de preferência pela sacarose. As análises neuroquímicas indicaram que os animais 6-OHDA-curcumina apresentaram níveis maiores de dopamina no estriado. Além disso, a curcumina também foi capaz de prevenir a morte dos neurônios dopaminérgicos da SNpc, como observado nas análises imunohistoquímicas. Em conclusão, estes resultados sugerem o efeito tipo-antidepressivo e neuroprotetor da curcumina no modelo animal da 6-OHDA de DP.



Palavras-chave: Doença de Parkinson, Curcumina, Depressão, 6-Hidroxidopamina.

## ABSTRACT

One of the most common nonmotor symptoms of Parkinson's disease (PD) is depression, which affects approximately 35% of patients and may significantly impair their quality of life. Curcumin, a major active compound of turmeric (*Curcuma longa*), has been found to have several pharmacological properties. It has antiinflammatory activity, inhibits monoamine oxidase (MAO), and has neuroprotective effects. This compound has already been extensively studied in various models of depression and, more recently, it has been studied in PD models. However, little is known about its effects on depression associated with PD. The present study investigated the antidepressant-like effect of curcumin in an animal model of 6-hydroxydopamine (6-OHDA)-induced PD. Male Wistar rats were randomly distributed into 4 groups (n=9-14/group): sham-vehicle; sham-curcumin; 6-OHDA-curcumin and 6-OHDA-vehicle. The rats received a bilateral intranigral infusion of 6-OHDA, and the sham group received vehicle. All of the rats were treated for 21 days with curcumin (30 mg/kg, p.o.) or vehicle (sunflower oil, p.o.) beginning 1 h after surgery. Twenty four hours and 21 days after surgery the animals were subjected to open field test. Immediately after, the rats were evaluated in the forced swim test (FST). For sucrose preference test, the procedures were performed before the surgeries and then weekly, starting 7 days after surgery until de 21 $^{th}$ day. The tyrosine hydroxylase (TH)-immunoreactivity of *substantia nigra pars compacta* (SNpc) neurons and the quantification of monoamines by HPLC were performed 21 days after the surgery. . 6-OHDA groups presented a reduction on motor parameters 1 day after surgery, which was reversed on 21$^{th}$ day. Curcumin exerted an antidepressant-like effect in both models of depression and increased the levels of dopamine and its metabolites in the striatum. Immunohistochemical analysis showed that curcumin was able to prevent the death of dopaminergic neurons in the SNpc. These findings suggest antidepressant-like and neuroprotective effects of curcumin in an animal model of 6-OHDA-induced PD.



Keywords: Parkinson's disease, Curcumin, Depression, 6-Hydroxydopamine.

**LISTA DE ABREVIATURAS**

5-HT – 5-hidroxitriptamina (Serotonina)
5-HIAA - Ácido 5-hidroxindol acético
6-OHDA – 6-hidroxidopamina
BDNF – Fator Neurotrófico Derivado do Encéfalo
BHE – Barreira Hematoencefálica
COX-1 – Ciclooxigenase 1
COX-2 – Ciclooxigenase 2
DA- Dopamina
DHPG- dihidroxifenilglicol
DOPAC - ácido 3,4-dihidroxifenilacético
DP – Doença de Parkinson
DPI – Doença de Parkinson Idiopática
HPLC – Cromatografia Líquida de Alta Performace
HVA - Ácido homovanílico
IL-6 - Interleucina-6
iNOS – Óxido Nítrico sintetase indutível
LCR – Líquido cefalorraquidiano
MAO - Monoaminooxidase
MAO-A - Monoaminooxidase tipo A
MAO-B - Monoaminooxidase tipo B
MPTP - 1-metil-4-fenil-1,2,3,6-tetraidropiridina
NA- Noradrenalina
NF-kB – Fator nuclear kappa-B
OH• - Radical hidroxila
PET- Tomografia por emissão de Pósitrons
ROS – Espécies reativas de oxigênio
SNC- Sistema Nervoso Central
SNpc – Substância negra parte compacta
TH – Tirosina Hidroxilase
TNF-α – Fator de necrose tumoral α
TNFm – Teste da Natação Forçada Modificado

**SUMÁRIO**

# 1. INTRODUÇÃO

## 1.1 DOENÇA DE PARKINSON

A Doença de Parkinson (DP) é uma doença neurodegenerativa caracterizada pela redução dos níveis de dopamina (DA) no estriado e na substância negra *pars compacta* (SNpc). Esta redução ocorre devido à perda dos neurônios dopaminérgicos da via nigroestriatal, responsável pela liberação da DA da SNpc para o corpo estriado (putâmen e caudado) (Figura 1 A,B). A morte desses neurônios resulta na redução do conteúdo de DA no estriado (Hornykiewicz, 2006; Dauer e Przedborski, 2003; Gerlach e Riederer, 1996), além da redução dos metabólitos estriatais: ácido homovanílico (HVA) e 3,4-dihidroxifenilacético (DOPAC). Em tecidos *post mortem* de pacientes parkinsonianos também foi verificado redução das enzimas responsáveis pela síntese de DA: tirosina hidroxilase (TH) e DOPA-descarboxilase (Lang e Lozano, 1998, Tabrez et al., 2012).

Estas alterações resultam no aparecimento dos sinais motores da DP que consistem em lentificação dos movimentos (bradicinesia), tremor muscular, rigidez e instabilidade postural (Dauer e Przedborski, 2003). Pacientes também apresentam sintomas vegetativos tais como movimento facial prejudicado, o qual interfere na fala e no piscar dos olhos, aumento de salivação, seborreia, constipação, vermelhidão e sudorese (Klockgether, 2004). Porém, estes sinais são observados somente após a perda de pelo menos 50% dos neurônios dopaminérgicos da SNpc e redução de aproximadamente 80% no conteúdo de DA na via nigroestriatal (Lang e Lozano,1998; Deumens et al., 2002).

Além dos sinais motores, os pacientes com DP também podem apresentar diversos sintomas não motores, que diminuem ainda mais a qualidade de vida desses pacientes. Dentre estes sintomas podem-se citar as disfunções olfatórias, os distúrbios do sono, os distúrbios gastrointestinais, o prejuízo cognitivo e déficit de atenção, a ansiedade, a demência e a depressão (Chaudhuri et al., 2006).

Na maioria dos casos da DP verificam-se os corpos de Lewy, que consistem em inclusões eosinofílicas tóxicas formadas por agregados proteicos de α-sinucleína ou ubiquitina (Figura 1.C) (Elbaz e Tranchant, 2007; Dauer e Przedorski,2003).

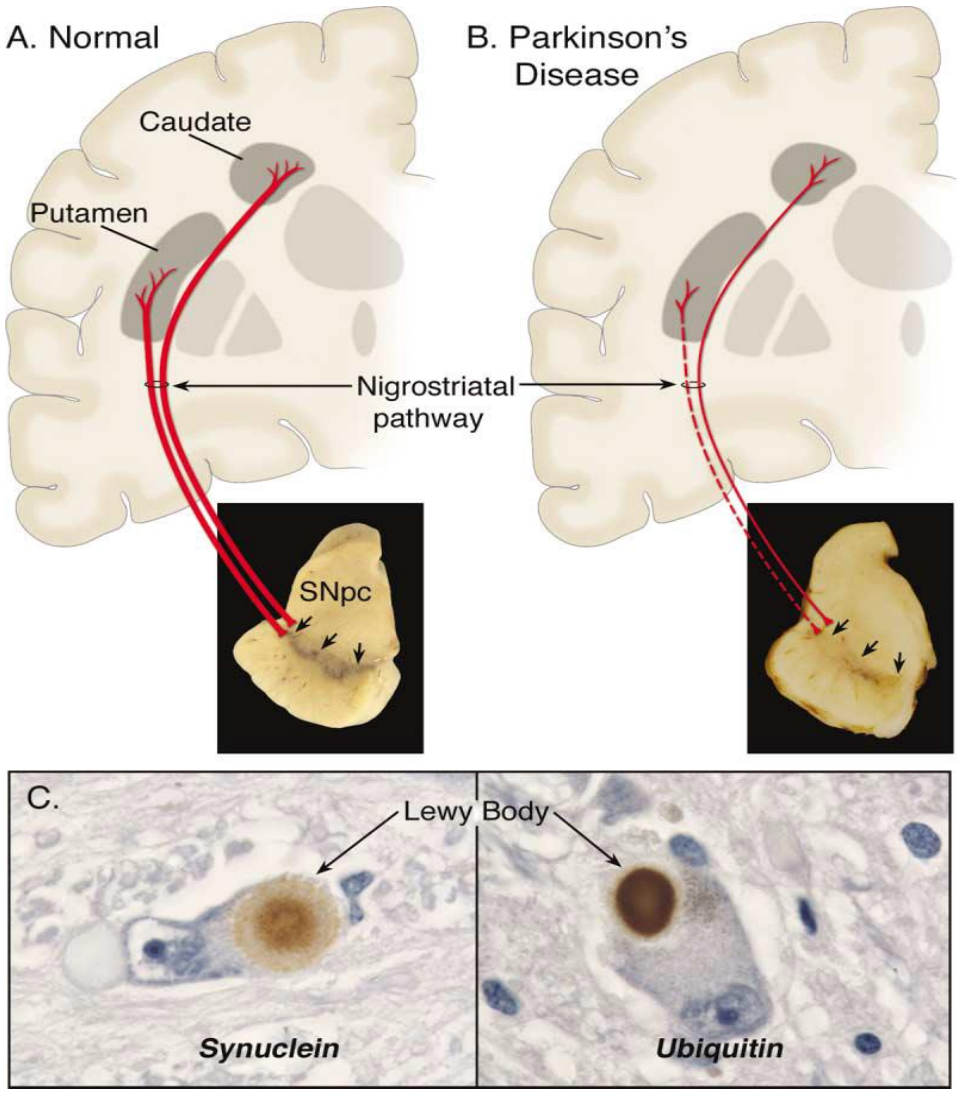


**Figura 1.** Morte neuronal e corpos de Lewy na Doença de Parkinson. (A) Esquema representando a via nigroestriatal normal e (B) de paciente com DP com despigmentação da SNpc. (C) Imunohistoquímica indicando presença de corpos de Lewy na SNpc. Fonte: Dauer e Przedorski,2003.

## 1.2 ETIOLOGIA DA DOENÇA DE PARKINSON

Existem dois principais tipos da DP, a familiar e a esporádica. A familiar, que acomete de 5 a 10% dos casos, surge de mutações em alguns genes como os da α-sinucleína e parkina (Pimentel,2009; Vila e Przedborski, 2004). Já a esporádica é dita como Doença de Parkinson Idiopática (DPI), ou seja, de causa desconhecida. Entretanto, apesar da diferença etiológica, ambos os tipos de DP são caracterizados pela neurodegeneração dopaminérgica da via nigroestriatal.

Apesar de não se saber exatamente a etiologia da DPI, sabe-se que a morte dos neurônios dopaminérgicos está ligada a uma série de fatores como disfunção mitocondrial (complexo I da cadeira respiratória), estresse oxidativo, neuroinflamação e aumento de excitotoxicidade (Esposito et al., 2007).

### 1.2.1 Estresse Oxidativo

O aumento do estresse oxidativo parece ter grande correlação com a morte neuronal na DP. O desequilíbrio entre a produção de espécies reativas de oxigênio (ROS) e os mecanismos antioxidantes endógenos podem levar a um aumento no estresse oxidativo que causará danos ao DNA, proteínas e lipídios e, consequentemente, morte celular por apoptose ou necrose. Regiões abundantes em catecolaminas são muito susceptíveis ao estresse oxidativo, destacando-se a metabolização da DA como geradora de ROS (Zhou et al., 2008; Mosley et al., 2006).

A DA pode ser metabolizada pela monoaminooxidase (MAO) ou ser auto-oxidada. Quando metabolizada pela MAO gera peróxido de hidrogênio ($H_2O_2$). Entretanto, quando auto-oxidada, além de $H_2O_2$, também ocorre a formação de dopamina-quinona. O $H_2O_2$ é facilmente reduzido para o radical hidroxila (OH•) na presença de $Fe^{2+}$, pela reação de Fenton. O OH•, por sua vez, tem grande poder oxidante e, assim como a dopamina-quinona, reage de maneira inespecífica com inúmeros componentes celulares alterando suas propriedades e funções. Sendo assim, estas espécies reativas são capazes de oxidar diversas proteínas, lipídios, enzimas e DNA levando ao dano e morte dos neurônios (Meiser et al., 2013; Mosley et al., 2006).

Estudos *post mortem* em pacientes parkinsonianos revelaram o aumento da peroxidação lipídica na SNpc destes indivíduos, indicando a ocorrência de estresse oxidativo (Meiser et al., 2013). Também já foi constatado que a SNpc possui níveis de ferro superiores a outras estruturas encefálicas, contribuindo para a redução do $H_2O_2$ para OH•. Ademais, estes níveis férricos são ainda superiores em pacientes com DP, dados que corroboram a importância do estresse oxidativo nesta patologia. (Hald e Lotharius, 2005).

### 1.2.2 Neuroinflamação e Doença de Parkinson

Dentre as diversas causas que levam a degeneração dos neurônios dopaminérgicos na DP, a neuroinflamação consiste em um dos principais mecanismos relacionados com essa patologia (McGeer et al., 2001). A micróglia corresponde a um grupo de células fagocíticas de defesa responsável por manter o funcionamento do sistema nervoso central (SNC) normal respondendo a infecções, inflamações e traumas (Kim e De Vellis, 2005; Beyer et al.,2000). A sensibilidade da micróglia frente a qualquer alteração no SNC é altíssima, com isso, esta população de células se torna ativa muito facilmente e acaba desencadeando um processo inflamatório no qual vai ativar diversos agentes pró-inflamatórios, neurotoxinas e moléculas de adesão (Orr et al.,2002). A micróglia também causa um aumento da expressão de enzima diretamente relacionadas ao processo inflamatório como a óxido nítrico sintetase induzível (iNOS) , ciclooxigenase-1 (COX-1) e ciclooxigenase-2 (COX-2) (Mosley et al., 2006). No caso de um estímulo inflamatório contínuo, os níveis de agentes pró-inflamatórios e moléculas de adesão se tornam exacerbados, levando a ativação da micróglia próxima dos neurônios dopaminérgicos, que levará a morte dos mesmos. A morte desses neurônios, por sua vez, ativará outro grupo de micróglia, que ativará mais agentes pró-inflamatórios e assim desencadear uma resposta inflamatória descontrolada levando a morte cada vez maior de neurônios (Kim e De Vellis, 2005; Aloisi, 2001; Banati et al.,1998; Bronstein et al.,1995).

Além disso, em estudos *post mortem* com pacientes com DP foi evidenciado o aparecimento da neuroinflamação. Nesses pacientes foi relatado uma maior reatividade da micróglia bem como uma maior expressão de diversas citocinas e enzimas inflamatórias como o fator nuclear kappa-B (NF-kB), fator de necrose tumoral α (TNF-α), interleucina-6 (IL-6), COX-1, COX-2 e iNOS. Todos esses dados sugerem a correlação entre o aumento do processo inflamatório e o desenvolvimento dessa patologia (Deleidi e Gasser, 2013; Banati et al.,1998; Mcgeer et al.,1988) .

## 1.3 MODELO ANIMAL DE DOENÇA DE PARKINSON

Para se estudar a etiologia, os mecanismos moleculares e as alternativas terapêuticas da DP se fez necessário o desenvolvimento de modelos animais desta doença. A partir disto, diversos estudos vêm sendo realizados a fim de se chegar a um modelo animal com maior similaridade à DP. Entretanto, ainda não se tem um modelo fidedigno desta enfermidade. Porém, através da administração de neurotoxinas específicas já se consegue reproduzir nos animais algumas características da DP, como a morte dopaminérgica nigroestriatal (Meredith et al., 2008; Dauer e Przedborski, 2003).

Dentre estes modelos animais, os mais utilizados e estudados são os de indução de parkinsonismo através da administração de 6-hidroxidopamina (6-OHDA), 1-metil-4-fenil-1,2,3,6-tetraidropiridina (MPTP) e rotenona. Todas estas neurotoxinas levam a morte dos neurônios dopaminérgicos através de mecanismos relacionados à inibição do complexo I da cadeia respiratória mitocondrial e aumento do estresse oxidativo (Figura 2) (Bové e Perier, 2011).

### 1.3.1 Modelo da 6-OHDA

O modelo da 6-OHDA foi o primeiro estudado para mimetizar a DP em animais e até hoje é um dos mais utilizados (Dauer e Przedborski, 2003). A 6-OHDA, por não conseguir atravessar a barreira hematoencefálica (BHE), tem que ser administrada diretamente no encéfalo, através de cirurgia estereotáxica. Sua administração pode se dar em diferentes regiões encefálicas como a SNpc, o estriado e o feixe prosencefálico medial. A extensão do dano causado pela infusão dessa neurotoxina bem como o tempo para a promoção da morte dos neurônios dopaminérgicos depende tanto da concentração como da região do encéfalo em que a 6-OHDA é administrada. Na SNpc, a morte dos neurônios dopaminérgicos se inicia 24 horas após a infusão da neurotoxina e causa extensa morte dopaminérgica, mimetizando um estágio avançado da DP (Meredith et al., 2008; Dauer e Przedborski, 2003).

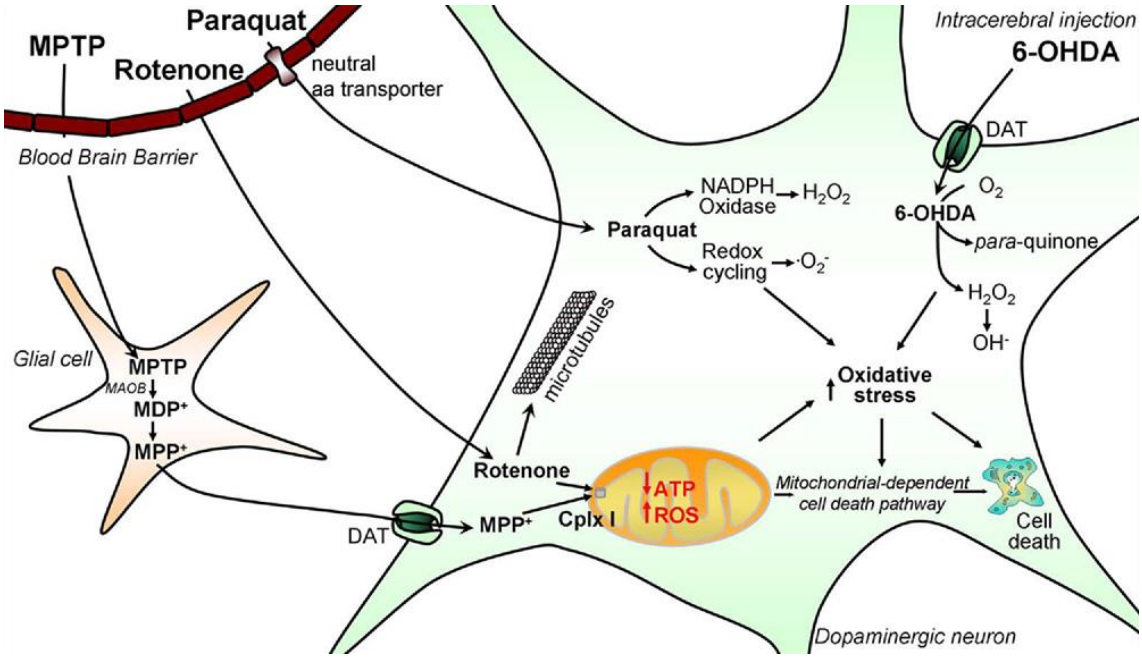


Figura 2. Mecanismos das neurotoxinas utilizadas como indutores de parkinsonismo. Representação dos mecanismos de promoção da morte de neurônios dopaminérgicos das principais neurotoxinas utilizadas para mimetizar a DP, MPTP, rotenona, paraquat e 6-OHDA. Fonte: Bové e Perier, 2011.

Devido a sua semelhança estrutural com a DA, a 6-OHDA consegue entrar nos neurônios dopaminérgicos através do transportador de catecolaminas (Bové e Perier, 2011; Meredith et al.,2008). O mecanismo pelo qual ela causa a morte neuronal consiste basicamente no aumento do estresse oxidativo. Quando a 6-OHDA entra no neurônio através dos transportadores de DA, ela é rapidamente auto-oxidada formando espécies reativas de oxigênio (ROS) como: quinonas, peróxido de hidrogênio e superóxido, levando a morte celular. Esta morte dos neurônios dopaminérgicos promove nos animais o aparecimento de alguns sinais presentes nos pacientes com DP, como prejuízo motor, alterações cognitivas e desenvolvimento de comportamento tipo-depressivo (Santiago et al., 2010; Tadaiesky et al., 2008).

## 1.4 DEPRESSÃO E DOENÇA DE PARKINSON

Um dos grandes problemas relacionados à depressão associada à DP é a realização de seu diagnóstico. Em ambas as patologias isoladas (DP e depressão maior) o indivíduo pode apresentar retardo psicomotor, redução da expressão facial, cansaço, redução do apetite e insônia ou hipersonia, sendo difícil diferenciar se estes sinais e sintomas estão ligados somente a DP propriamente dita ou agregados à depressão (Dissanayaka et al., 2011). Mesmo com esta dificuldade de diagnóstico, a depressão é um dos sintomas não motores mais frequentes da DP, acometendo cerca de 35% dos pacientes **(**Aarsland et al., 2012). Pacientes que apresentam um quadro depressivo associado à DP possuem uma qualidade de vida ainda inferior aqueles não acometidos por este sintoma. Além do mais, o aparecimento do sintoma depressivo na DP acarreta em um aumento significativo no custo do tratamento dessa patologia (Dissanayaka et al., 2011).

A depressão associada à DP está envolvida com diversos fatores. O estresse psicossocial, causado pelo diagnostico de uma patologia progressiva, sem cura e desencadeante de limitações motoras e cognitivas, pode levar o paciente a um quadro depressivo. Além do mais, a própria neurodegeneração característica da doença, que acomete não somente neurônios dopaminérgicos como também neurônios serotoninérgicos, noradrenérgicos e colinérgicos, leva redução dos níveis de monoaminas, processo diretamente relacionado com a depressão maior (Wolters, 2008; Cumming e Masterman, 1999; Mayeux, 1990).

Embora etiologia da depressão-DP ainda não esteja totalmente elucidada, sabe-se que inúmeras vias estão relacionadas com esse processo. Na depressão maior, além a disfunção monoaminérgica, a neuroinflamação e a neurodegeneração apresentam um importante papel na fisiopatologia dessa doença (Hurley e Tizabi, 2013). Na depressão associada à DP diversos mediadores inflamatórios estão aumentados, fator que pode resultar em um processo inflamatório que contribuiria com a morte neuronal (Aarsland et al., 2012; Barnum e Tansey, 2012; Gao et al., 2002).

Apesar das evidências da importância da serotonina (5-HT) na depressão maior, o papel desse neurotransmissor na depressão associada à DP ainda não está totalmente elucidado. Devido a estudos inconclusivos abordando o

tratamento da depressão-DP utilizando antidepressivos convencionais, inibidores seletivos da receptação de 5-HT, e o fato que a depleção aguda de triptofano, precursor da 5-HT, não leva ao aparecimento de sintomas depressivos em pacientes com DP sem depressão, o envolvimento de outras monoaminas parece estar ligado ao surgimento desse sintoma não motor (Skapinakis et al., 2010; Leentjens et al., 2006).

Entretanto, alterações nas vias dopaminérgicas e noradrenérgicas parecem apresentar correlação com aparecimento de sintomas depressivos. Pacientes com DP apresentam redução na neurotransmissão dopaminérgica, a qual está ligada aos sistemas de recompensa e humor. Em pacientes com DP, sabe-se há uma grande degeneração do *locus coeruleus*, a maior estrutura noradrenérgica do encéfalo. Porém esta degeneração é ainda maior em pacientes com depressão associada à DP do que naqueles que não apresentam o estado depressivo (Aarsland et al., 2012). Também foi constatado, em estudos com pacientes com DP, utilizando tomografia por emissão de pósitrons (PET), uma associação entre a redução de transportadores dopaminérgicos e noradrenérgicos na região límbica e do estriado ventral e a incidência de depressão (Remy et al., 2005), indicando que talvez estes sejam os principais neurotransmissores envolvidos no aparecimento dos sintomas depressivos na DP (Aarsland et al., 2012).

## 1.5 CURCUMINA

A *Curcuma longa* L.*,* planta de origem asiática popularmente conhecida como açafrão-da-índia, cúrcuma, batata-amarela, gengibre-dourada, açafrão-da-terra ou “tumeric”, é amplamente utilizada na dieta em países asiáticos estando presente nas mais diversas culinárias e temperos, sendo utilizada principalmente como corante devido sua coloração amarelo-alaranjada (Darvesh et al., 2012; Rosa, 2009). O principal responsável pela coloração intensa extraída da *Curcuma longa* L. é a curcumina. A curcumina, uma molécula de diferuloimetano (Figura 3), é um flavonóide que possui diversas propriedades farmacológicas (Guerra-Araiza et al., 2013; Darvesh et al., 2012).

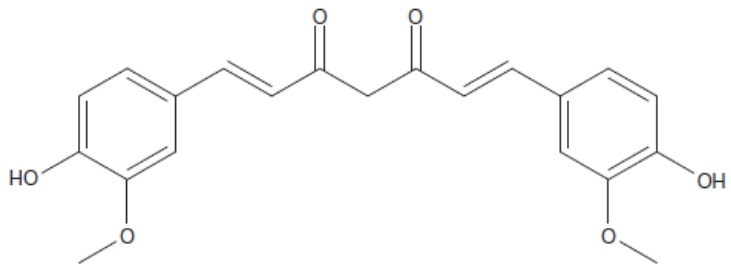


**Figura 3.** Estrutura química da curcumina. Fonte Darvesh et al., 2012.

Entre estas propriedades farmacológicas se destacam o seu potencial antioxidante, anti-inflamatório e neuroprotetor (Lopreseti et al., 2012; Jaques et. al., 2011). Em relação a sua atividade anti-inflamatória e antioxidante, a curcumina modifica a expressão de inúmeras proteínas, enzimas e citocinas, como interleucinas, fatores de necrose tumoral, COX-2, iNOS, entre outros, sempre reduzindo a resposta inflamatória e protegendo o meio contra ROS (Aggarwal e Harikumar, 2009).

Em modelos de depressão foram constatados o efeito tipo-antidepressivo deste composto, tanto em testes comportamentais como em bioquímicos. A curcumina se mostrou capaz de aumentar os níveis noradrenalina (NA), 5-HT e DA no córtex frontal, hipocampo (5-HT e DA) e estriado (DA) (Xu et al., 2005), além de aumentar os níveis de fator neurotrófico derivado do cérebro (BDNF) promovendo a neurogênese hipocampal, (Hurley et al., 2013) e inibir enzimas como monoaminooxidase tipo A (MAO-A) e monoaminooxidase tipo B (MAO-B), responsáveis pela metabolização das monoaminas (Kulkarni et. al. 2009).

Em relação à DP, a grande maioria dos estudos realizados atualmente foram estudos *in vitro*, nos quais foi possível observar efeitos neuroprotetores contra toxinas como MPTP e 6-OHDA (Rajeswari e Sabesan, 2008; Wang et al., 2009 e Yu et al., 2010). Mais ainda, foi verificado que a curcumina foi capaz de se ligar a α-sinucleína prevenindo a agregação e consequentemente a formação de corpos de Lewy, característicos na DP (Ahmad e Lapidus, 2012; Pandey et al., 2008). Dentre os estudos *in vivo,* foram observados efeitos preventivos e neuroprotetores da curcumina, uma vez que a administração da

mesma se iniciou antes da indução do parkinsonismo. Agrawal et al. (2012) constataram que animais pré-tratados por 21 dias com curcumina (60 mg/kg,v.o.) com posterior infusão de 6-OHDA mostraram maior expressão de neurônios TH-positivos na SNpc e níveis mais elevados de DA, DOPAC e HVA no estriado quando comparados aos animais lesionados tratados com veículo, indicando o efeito protetor da curcumina. Em estudos em que o tratamento com curcumina se iniciou imediatamente após a exposição dos animais a toxinas, foi observado o efeito da curcumina em prevenir a morte dos neurônios dopaminérgicos por inibição da resposta glial no estriado e na SNpc (Tripanichkul e Jaroensuppaperch, 2012). Dentre os mecanismos pelos quais a curcumina exerce seu efeito protetor contra citotoxicidade da 6-OHDA destacam-se efeitos antioxidantes e de moduladores do NF-kB (Wang et al., 2009).

Surpreendentemente, até este momento, não foi encontrado na literatura dados relacionando a curcumina com a depressão associada à DP. Deste modo, o presente estudo investigou os efeitos tipo-antidepressivos e neuroprotetores da curcumina no modelo animal de parkinsonismo induzido por 6-OHDA.

## 2. OBJETIVO GERAL

Avaliar o efeito tipo-antidepressivo da curcumina em modelo animal de Doença de Parkinson induzida por 6-OHDA.

### 2.1 OBJETIVOS ESPECÍFICOS

- Avaliar a indução de comportamento tipo-depressivo em ratos, através da infusão de 6-OHDA, no teste de natação forçada modificado (TNFm) e a capacidade de reversão deste efeito pelo tratamento com curcumina por 21 dias;
- Investigar a indução da anedonia pela infusão intranigral de 6-OHDA e a reversão deste quadro pelo tratamento prolongado com curcumina, através do teste de preferência pela sacarose;
- Verificar, utilizando o teste do campo aberto, a capacidade de reversão da hipolocomoção causada pela infusão de 6-OHDA nos animais através da administração aguda de curcumina;
- Quantificar os níveis de 5-HT, NA e DA e seus metabólitos, pela técnica de cromatografia líquida de alta performance (HPLC), no hipocampo e estriado e relacionar estes resultados com os obtidos nos testes comportamentais;
- Quantificar, através da técnica de imunohistoquimica com marcação para TH, a morte dos neurônios dopaminérgicos na SNpc causada pela infusão intranigral de 6-OHDA e verificar a capacidade de proteção contra a morte neuronal através do tratamento prolongado com curcumina;

**3.**

# Antidepressant-like effect of curcumin in 6-hydroxydopamine model of Parkinson's disease

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**Abstract**

One of the most common nonmotor symptoms of Parkinson's disease (PD) is depression, which affects approximately 35% of patients and may significantly impair their quality of life. Curcumin, a major active compound of turmeric (*Curcuma longa*), has been found to have several pharmacological properties. It has antiinflammatory activity, inhibits monoamine oxidase (MAO), and has neuroprotective effects. This compound has already been extensively studied in various models of depression and, more recently, it has been studied in PD models. However, little is known about its effects on depression associated with PD. The present study investigated the antidepressant-like effect of curcumin in an animal model of 6-hydroxydopamine (6-OHDA)-induced PD. Male Wistar rats received a bilateral intranigral infusion of 6-OHDA, and the sham group received vehicle. All of the rats were treated for 21 days with curcumin (30 mg/kg, p.o.) or vehicle (sunflower oil, p.o.) beginning 1 h after surgery. The animals were subjected to the forced swim test and sucrose preference test. Curcumin exerted an antidepressant-like effect in both tests. Neurochemical analyses were then performed. Curcumin increased the levels of dopamine and its metabolites in the striatum. Immunohistochemical analysis showed that curcumin was able to prevent the death of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc). These findings suggest antidepressant-like and neuroprotective effects of curcumin in an animal model of 6-OHDA-induced PD.

**Keywords:** Parkinson's disease, Curcumin, Depression, 6-Hydroxydopamine

## Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by dopaminergic neuron loss in the substantia nigra *pars compacta* (SNpc) (Gerlach et al.,1996; Dauer and Przedborski, 2003; Hornykiewicz, 2006). Parkinson's disease patients present a large number of motor impairments associated with this disease, such as bradykinesia, tremor at rest, postural instability, and freezing (Dauer and Przedborski, 2003). However, these patients may also develop nonmotor symptoms, including olfactory problems, constipation, sleep disorders, anxiety, dementia, and depression (Chaudhuri et al., 2006). Depression associated with PD occurs in approximately 35% of PD cases (Aarsland et al., 2012). Depression in PD is related to psychosocial stress caused by the disease and the neurodegeneration process (Schrag 2004; Tadaiesky et al., 2008; Santiago et al., 2010). This neurodegeneration in PD is mainly associated with dopaminergic neurons, but serotoninergic, noradrenergic, and cholinergic systems are also involved (Wolters, 2008; Aarsland et al., 2012). The precise etiology of depression in PD has not been elucidated, but numerous pathways have been linked to this process. In major depression, monoaminergic dysfunction, neuroinflammation, and neurodegeneration play important roles in the disease's pathophysiology (Hirsch et al., 2013; Zunszain et al., 2013; Hurley and Tizabi, 2013). Depression associated with PD appears to be associated with an increase in inflammation pathways, in which several inflammatory mediators are upregulated (Aarsland et al., 2012). This increase could result from an uncontrolled process that may contribute to neuronal death (Gao et al., 2002). Despite the importance of serotonin (5-hydroxytrypatamine [5-HT]) in major depression, the role of this

neurotransmitter in PD-related depression has not been elucidated (Aarsland et al., 2012). Studies have been inconclusive with regard to treating PD-related depression with conventional antidepressant drugs that inhibit 5-HT reuptake. The depletion of tryptophan (i.e., the 5-HT precursor) did not promote depressive symptoms in PD patients without depression, suggesting that other monoamines could be more involved in the emergence of this PD symptom (Leentjens et al., 2006; Skapinakis et al., 2010). Parkinson's disease patients present impairments in dopaminergic pathways related to reward and mood systems. A reduction of dopamine (DA) levels in frontal and subcortical regions has been observed in PD patients with depression, thus linking this neurotransmitter with PD-related depression. Additionally, norepinephrine (NE) appears to play an important role in the emergence of PD-related depression. Depressive PD patients presented greater neurodegeneration in the locus coeruleus, a structure linked to noradrenergic function, compared with non-depressive PD patients (Aarsland et al., 2012).

Curcumin is a major active compound of *Curcuma longa* L. It is a molecule with multiple pharmacological properties, with antioxidant, antiinflammatory, and neuroprotective effects (Jaques et al., 2011; Lopresti et al., 2012). Curcumin has been shown to exert an antidepressant-like effect based on behavioral and biochemical analyses in depression models. Curcumin enhanced monoamine levels, promoted hippocampal neurogenesis by increasing brain-derived neurotrophic factor levels, and inhibited monoamine oxidase (MAO), an enzyme linked to monoamine metabolism (Xu et al., 2005a; Kulkarni et al., 2009; Hurley et al., 2013). Most studies that have used PD models have been performed *in vitro*. The neuroprotective effects of curcumin

were observed against toxins, such as MPTP and 6-OHDA (Rajeswari e Sabesan, 2008; Yu et al., 2010). *In vitro* studies showed that curcumin is able to bind α-synuclein to prevent its aggregation and consequently Lewy body formation (Pandey et al., 2008; Ahmad and Lapidus, 2012). *In vivo* studies also showed that pretreatment with curcumin protected against dopaminergic neuron death caused by toxin administration in the SNpc (Agrawal et al., 2012). Studies that began curcumin treatment immediately after toxin exposure found that it inhibited MAO-B in the striatum (Rajeswari e Sabesan, 2008). Curcumin was also shown to prevent dopaminergic neuron death by inhibiting the glial activation response in the striatum and SNpc (Tripanichkul and Jaroensuppaperch, 2012).

To date, little is known about the effects of curcumin in PD-related depression. Given the multiple mechanisms of action of curcumin and the multifactorial pathways of depression in PD (Lopresti et al., 2012), we investigated whether curcumin would be effective in an animal model of PD-related depression. We injected 6-OHDA bilaterally into the SNpc and evaluated whether curcumin could protect against the depressive-like effect of the neurotoxin. To support the behavioral studies, neurochemical and immunohistochemical analyses were also performed.

## Methods

### *Animals*

Male Wistar rats, 290-320 g, were used. They were randomly housed under standard conditions of temperature (22 ± 2ºC) and illumination (12 h/12 h light/dark cycle). They had free access to water and food throughout the

experiment. The studies were performed in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals, United States National Institutes of Health. The protocol complied with the recommendations of the Federal University of Paraná and was approved by the Institutional Ethics Committee (protocol no. 590).

*Experimental design*

The animals were randomly distributed into the following four groups (*n* = 9-14/group): sham-vehicle, sham-curcumin, 6-OHDA-vehicle, and 6-OHDA-curcumin. All of the rats underwent stereotaxic surgery. One hour after surgery when the rats recovered from anesthesia, the treatment began, ending 21 days later. The animals orally received 30 mg/kg curcumin (Sigma, St. Louis, MO, USA) or vehicle (sunflower oil) daily between 9:00 AM and 10:00 AM. The curcumin dose was based on a pilot study performed in our laboratory (unpublished data). Three different doses of curcumin (10, 20, and 30 mg/kg, p.o.) were tested in an acute administration protocol. After three administrations (24, 8, and 1 h before the test), the rats were subjected to the forced swim test. Although all three curcumin doses exerted antidepressant-like effects, the 30 mg/kg dose was the most significant and similar to imipramine treatment.

The study was divided into two parts. In both experiments, the animals received the same treatment schedule as described above. In Experiment 1 (Fig. 1 A) (*n* = 10-12/group), all of the rats were subjected to the open field test 24 h and 21 days after stereotaxic surgery. On day 21, the training session of the forced swim test was conducted. Twenty-four hours later, the test session of the forced swim test was performed.

In Experiment 2 (Fig 1B) (*n* = 12-14/group), all of the rats were subjected to the sucrose preference test prior to surgery to determine the basal values of sucrose preference. Rats with sucrose preference < 75% were discarded from the study. Rats with sucrose preference > 75% underwent stereotaxic surgery. The sucrose preference test was performed 7, 14, and 21 days after surgery. After the last sucrose preference test, some of the rats (*n* = 9-10/group) were immediately decapitated, and the striatum and hippocampus were dissected. The remaining animals (*n* = 3-4/group) were submitted to a perfusion process for immunohistochemistry.

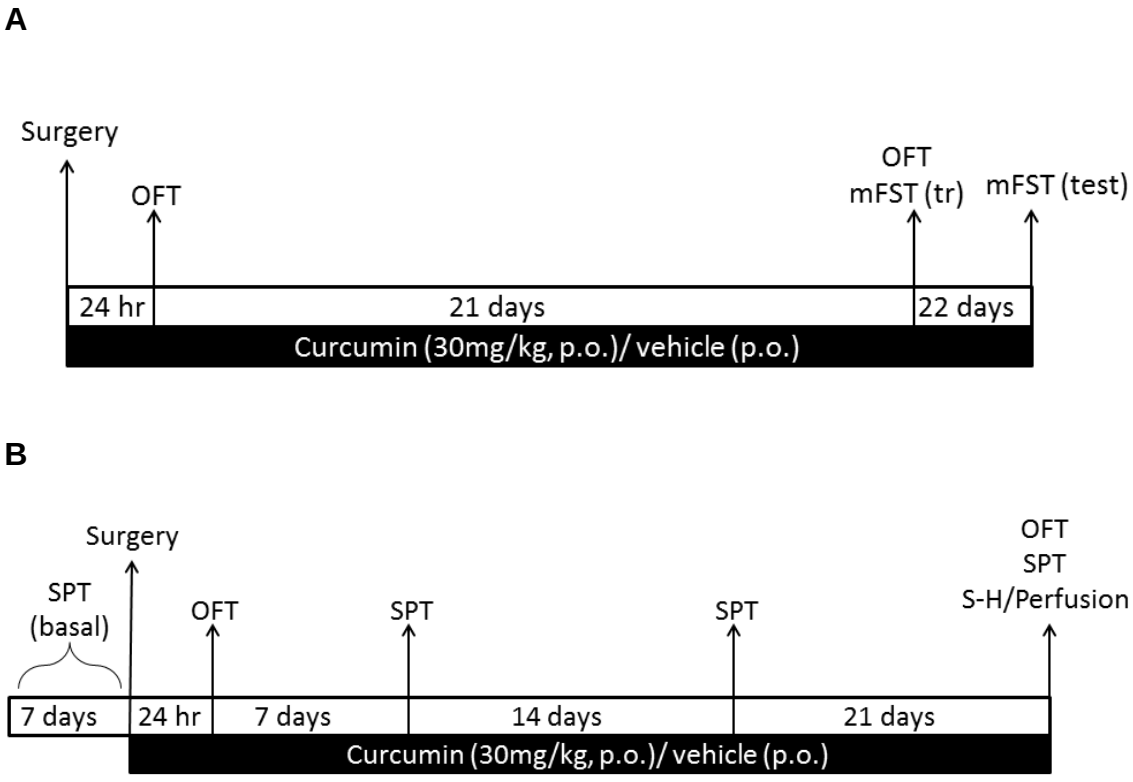



**Figure 1.** Experimental design. Experiment 1 (A) and experiment 2 (B). OFT-open field tes; mFST (tr) – modified forced swim test training session; mFST(test) – modified forced swim test test session; SPT – sucrose preference test; S-H – dissection of *striatum* and *hippocampus*.

*Stereotaxic surgery*

6-OHDA was infused into the SNpc during stereotaxic surgery. All of the animals were anesthetized with equitesin (chlornembutal, 0.3 ml/kg, intraperitoneal—i.p.), and 6-OHDA was bilaterally infused (6 µg in 1 µl of cerebrospinal fluid added to 0.2% ascorbic acid) using a 27-gauge needle attached to a 10 µl syringe (Hamilton, USA). The following coordinates were used for the SNpc: anterior/posterior, -5.0 mm from bregma; medial/lateral, ±2.1 mm from the midline; dorsal/ventral, 8 mm from the skull (Paxinos and Watson, 2005). To prevent reflux of the neurotoxin, the needle was kept at the infusion site for 2 min after completion of the procedure. The sham groups also underwent the same procedure but received artificial cerebrospinal fluid instead of 6-OHDA.

*Open field test*

The apparatus consisted of a circular, 100 cm diameter, 45 cm high arena, with the floor divided into 19 units. The animals were gently placed in always the same unit of the open field and allowed to freely explore the arena for 5 min. Two motor parameters were recorded throughout this test: locomotion frequency (i.e., the number of crossings from one unit to another) and rearing frequency (i.e., the number of times the animals stood on their hind paws). The open field was washed with a 5% water-ethanol solution before the behavioral tests to eliminate possible bias caused by odors left by previous rats. This test was performed according to Experiment 1, 24 h and 21 days after stereotaxic surgery.

*Modified forced swim test*

The modified forced swim test is often used to screen antidepressant drugs (Cryan et al., 2002). This test was performed 22 days after stereotaxic surgery. The animals were subjected to a 15 min training session on day 21. They were placed in a 25 cm diameter, 60 cm high tank that contained water at a temperature of 24 ± 1ºC and depth of 25 cm. Twenty-four hours prior to the training session, the rats were subjected to the forced swim test for 5 min. The entire experiment was filmed, and immobility, climbing, and swimming parameters were analyzed and quantified. After each animal, the water was changed to avoid any influence. This procedure is a modification (Reneric et al., 2002) of the method proposed by Porsolt et al. (1978).

*Sucrose preference test*

To verify anhedonia-like behavior, the sucrose preference test was performed (Papp et al., 1991). The rats were isolated and exposed to two pre-weighed bottles (one with water and one with 1% sucrose solution) for 24 h. Fluid consumption was quantified by calculating the difference in the weights of the bottles after 24 h (% sucrose preference = sucrose intake $\times$ 100 / total intake). The experiment was conducted before surgery to obtain baseline values and subsequently performed weekly until day 21, always between 9:00 AM and 10:00 AM. Animals with sucrose preference < 75% in the baseline assessment were discarded from the study.

*Determination of dopamine, norepinephrine, serotonin, and metabolite concentrations*

We used reverse-phase high-performance liquid chromatography (HPLC) with electrochemical detection to measure the levels of DA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), NE, dihydroxyphenylglycol (DHPG), 5-HT, and 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus and striatum. On day 21, the rats were decapitated, and encephalic structures were dissected and stored at −80ºC until processed for neurochemical quantification. The HPLC system consisted of a Synergi Fusion-RP C-18 reverse-phase column (150 mm × 4.6 mm inner diameter, 4 µm particle size) fitted with a 4 mm × 3.0 mm pre-column (Security Guard Cartridges Fusion-RP), an electrochemical detector (ESA Coulochem III Electrochemical Detector) equipped with a guard cell (ESA 5020) with the electrode set at 350 mV, a dual-electrode analytical cell (ESA 5011A), and a LC-20AT pump (Shimadzu) equipped with a manual Rheodyne 7725 injector with a 20 µl loop. A 25ºC control temperature was maintained inside the column. The cell contained two chambers in series. Each chamber included a porous graphite coulometric electrode, a double counter electrode, and a double reference electrode. Oxidizing potentials were set at 100 mV for the first electrode and 450 mV for the second electrode. The tissue samples were homogenized with an ultrasonic cell disrupter (Sonic) and 0.1 M perchloric acid that contained 0.02% sodium metabisulfite and an internal standard. After centrifugation at 10,000 × *g* for 30 min at 4ºC, 20 µl of the supernatant was injected into the chromatograph. The mobile phase, with a flow rate of 1 ml/min, had the following composition: 20 g citric acid monohydrate (Merck), 200 mg octane-1-sulfonic acid sodium salt

(Merck), 40 mg ethylenediaminetetraacetic acid (EDTA; Sigma), and 900 ml HPLC-grade water. The pH of the buffer running solution was adjusted to 4.0, and the solution was filtered through a 0.45 µm filter. Methanol (Merck) was added to give a final composition of 10% methanol (v/v). The neurotransmitter and metabolite concentrations were calculated using standard curves that were generated by determining the ratios between three different known amounts of the internal standard in triplicate. The units are expressed as µg/g of wet weight.

*Immunohistochemistry*

Midbrain dopaminergic cells were plotted using tyrosine hydroxylase (TH) immunohistochemical stain according to Reksidler et al. (2007). After the animals were anesthetized with thiopental, saline was intracardially infused, followed by a 4% paraformaldehyde fixative solution. Subsequently, the brains were removed and immersed in fixative solution for 3 days at 4ºC. Paraformaldehyde was changed daily until the third day of the procedure. During the last 3 days, the brains were placed in a 30% sucrose solution for 48 h. Then, the brains were stored in a freezer (-80° C). That done, they were fixed in a cryostat at -20 °C and cut. From the corpus callosum 180 cuts was counted to reach the substantia nigra, according to Paxinos and Watson, 1998. So were collected 12 cuts in the SNpc to 40 uM and placed on plates containing substance anti freezing.

The slices were washed three times with 0.1 M phosphate-buffered saline (PBS) solution for 10 min each. Endogenous peroxidase was blocked with a $H_2O_2$ + $H_2$Od solution for 10 min. The sections were washed again with 0.1 M PBS for 10 min, and the reaction was stopped with blocking buffer (30 ml

of 0.1 M PBS, 84 µl Triton X-100, and 450 µl normal goat serum). After blocking, the slices were incubated overnight in a solution that contained the primary antibody anti-TH. The next day, the sections were washed five times for 5 min each with 0.1 M PBS solution and subsequently incubated for 2 h with the secondary antibody biotin. After 2 h, the slices were washed again three times for 10 min each with 0.1 M PBS and then incubated in a solution of Complex AB for 2 h. The slices were washed again with 0.1 M PBS for 10 min, and the samples were incubated with vectorstain (DAB) for 6-8 min. Finally, the slices were washed with 0.1 M PBS (three times for 5 min each and three times for 10 min each). The sections were mounted on gelatin-coated slides and dried for 48 h. The slices were dehydrated in alcohol and cleared in xylene (5 min for each solution). The slides were then covered with Entellan, coverslipped, and analyzed using an optical microscope by comparing the number of TH-positive cells in the SNpc in all of the groups.

*Statistical analysis*

Differences between groups in the open field test and forced swim test were analyzed using one-way analysis of variance (ANOVA) followed by the Newman-Keuls *post hoc* test. The sucrose preference test data were analyzed using two-way ANOVA followed by the Bonferroni *post hoc* test. The two factors were time and treatment. The neurochemical and histological data were analyzed using one-way ANOVA followed by the Newman-Keuls test. The data are expressed as mean ± standard error of the mean (SEM). The level of significance was set at $p \leq 0.05$.

## Results

*Open field test*

With regard to motor activity (Table 1) 24 h after stereotaxic surgery, rearing frequency ($F_{3,40}$ = 7.769, $p$ = 0.0003) and locomotion frequency ($F_{3,40}$ = 11.49, $p$ < 0.0001) were reduced in the 6-OHDA groups compared with sham rats ($p$ < 0.01). In the second measure performed on day 21, no differences in rearing frequency ($F_{3,36}$ = 0.07918, $p$ = 0.9709) or locomotion frequency ($F_{3,36}$ = 0.2508, $p$ = 0.8603) were observed between groups.

**Table 1.** Motor behavior alterations determined 1 and 21 days after 6-OHDA exposure

| | Group | Locomotion Frequency | Rearing Frequency |
|---|---|---|---|
| Day 1 | Sham-vehicle | 99.27 ± 7.58 | 21.27 ± 1.95 |
| | Sham-curcumin | 110.1 ± 11.52 | 18.36 ± 2.03 |
| | 6-OHDA-vehicle | 49.42 ± 14.54* | 9.5 ± 3.19* |
| | 6-OHDA-curcumin | 28.9 ± 9.38** | 6.5 ± 2.42* |
| Day 21 | Sham-vehicle | 79.82 ± 9.02 | 15.45 ± 2.44 |
| | Sham-curcumin | 67.18 ± 13.76 | 15.22 ± 2.13 |
| | 6-OHDA-vehicle | 69.20 ± 10.21 | 16.78 ± 3.11 |
| | 6-OHDA-curcumin | 71.63 ± 12.7 | 16.11 ± 1.95 |

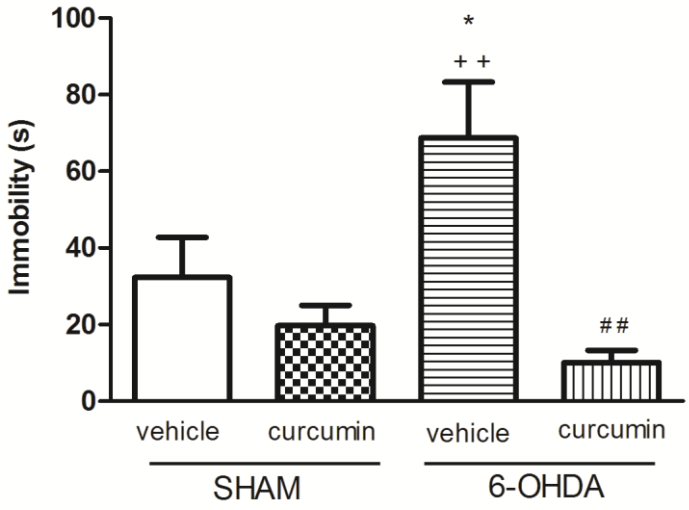
The data are expressed as mean ± SEM ($n$ = 10-12/group). *$p$ < 0.01, **$p$ < 0.001, compared with sham groups (ANOVA followed by Newman-Keuls test).

*Modified forced swim test*

The 6-OHDA-vehicle group showed an increase in immobility time ($F_{3,30}$ = 7.237, $p$ = 0.001) compared with the sham groups (sham-vehicle, $p$ < 0.05; sham-curcumin, $p$ < 0.01). The 6-OHDA-curcumin group exhibited a reduction of this parameter compared with the 6-OHDA-vehicle group ($p$ < 0.01; Fig. 2A). The swimming parameters ($F_{3,30}$ = 8.056, $p$ = 0.0004) showed that the 6-OHDA-

curcumin group exhibited an increased swimming time compared with the 6-OHDA-vehicle group ($p < 0.01$). A reduction of swimming time was observed in the 6-OHDA-vehicle group compared with the sham-vehicle group ($p < 0.05$) and sham-curcumin group ($p < 0.001$). In contrast, the sham-curcumin group exhibited an increase in this parameter compared with the sham-vehicle group ($p < 0.05$; Fig. 2B). Climbing time ($F_{3,30} = 1.667$, $p = 0.1952$) did not significantly differ between groups (Fig. 2C).

**A**

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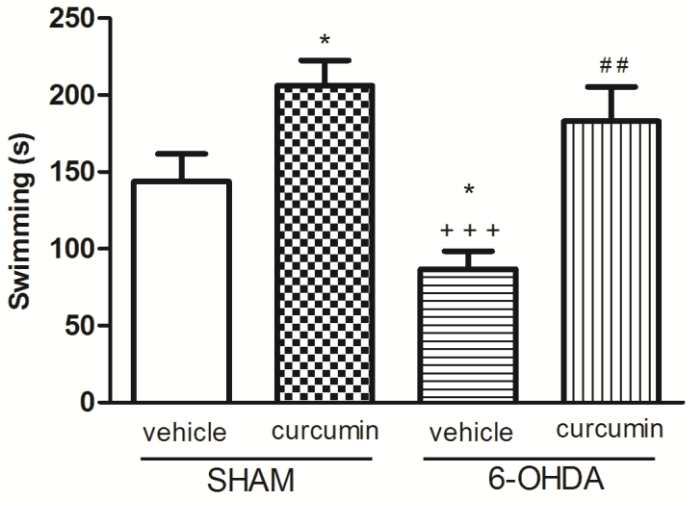

**C**

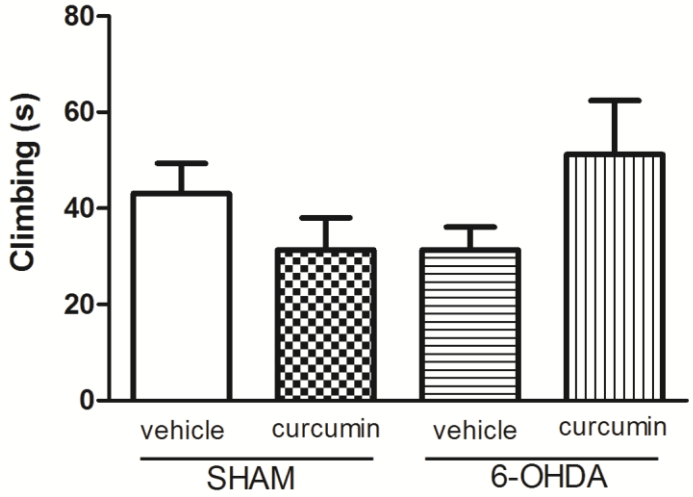


**Figure 2.** Antidepressant-like effect of curcumin (30 mg/kg, p.o.) and depressive-like behavior caused by 6-OHDA injection in the forced swim test. (A) Immobility. (B) Swimming. (C) Climbing. The data were obtained 22 days after neurotoxin exposure. The data are expressed as mean ± SEM ($n$ = 10-12/group). *$p < 0.05$, compared with sham-vehicle group; $^{\#\#}p < 0.01$, compared with 6-OHDA-vehicle group; $^{++}p < 0.01$, $^{+++}p < 0.001$, compared with sham-curcumin group (ANOVA followed by Newman-Keuls test).

*Sucrose preference test*

As shown in Fig. 3, sucrose consumption in the 6-OHDA-vehicle group decreased compared with the 6-OHDA-curcumin, sham-vehicle, and sham-curcumin groups on day 14 ($p < 0.001$). Sucrose preference in the 6-OHDA-vehicle group remained lower than in the sham groups ($p < 0.01$) on day 21. No difference was observed between the sham groups and 6-OHDA-curcumin group at any of the time-points.

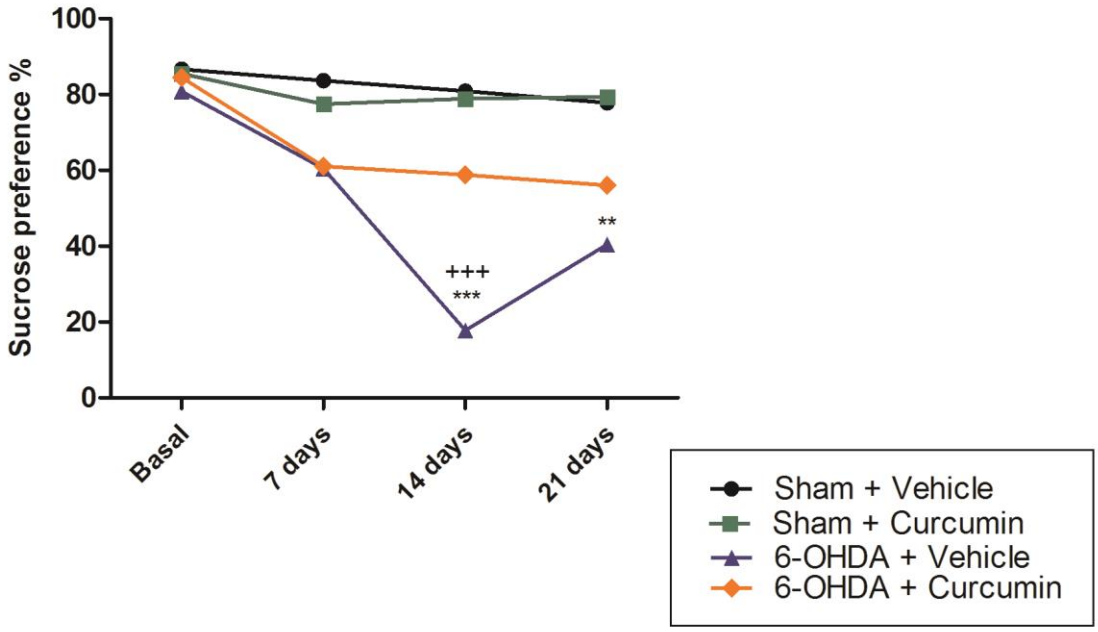


**Figure 3**. Antidepressant-like effect of curcumin (30 mg/kg, p.o.) and anhedonic-like behavior caused by 6-OHDA injection, reflected by the percentage of sucrose preference in each group at different time-points. The data are expressed as the mean ($n$ = 12-14/group). **$p$ < 0.01, ***$p$ < 0.001, compared with sham groups; $^{+++}p$ < 0.001, compared with 6-OHDA-curcumin group (two-way ANOVA followed by Bonferroni test).

*Determination of dopamine, norepinephrine, serotonin, and metabolite concentrations*

Striatal DA concentrations ($F_{3,37}$ = 9.805, $p$ = 0.0002) decreased in the 6-OHDA-vehicle group compared with both sham groups ($p$ < 0.001). The 6-OHDA-curcumin group exhibited an increase in DA concentrations compared with the 6-OHDA-vehicle group ($p$ < 0.01). However, DA levels in the striatum in the 6-OHDA-curcumin group decreased compared with the sham groups ($p$ < 0.01). A significant difference was observed between the sham groups ($p$ < 0.05), when animals treated with exhibited an increase in DA concentrations (Table 2). The concentrations of DOPAC ($F_{3,37}$ = 10.60, $p$ < 0.0001) and HVA ($F_{3,37}$ = 9.805, $p$ = 0.0002) in the striatum exhibited the same profile. In the 6-

OHDA-vehicle group, the metabolite concentrations were reduced compared with the sham groups ($p < 0.001$). Interestingly, no difference was found between the 6-OHDA-vehicle and 6-OHDA-curcumin groups. Reductions of DOPAC and HVA concentrations were observed in the 6-OHDA-curcumin group compared with the sham groups ($p < 0.05$; Table 2).

The 6-OHDA-vehicle group exhibited a significant reduction of hippocampal NE levels ($F_{3,37} = 11.58$, $p < 0.0001$) compared with the sham groups ($p < 0.001$; Table 3). The 6-OHDA-curcumin group exhibited a decrease in NE concentration in the hippocampus compared with the sham-vehicle group ($p < 0.01$) and sham-curcumin group ($p < 0.001$).

The concentrations of 5-HT ($F_{3,37} = 0.6210$, $p = 0.6059$) and 5-HIAA ($F_{3,37} = 0.3746$, $p = 0.7718$) in the hippocampus were not significantly different between groups (Table 3).

**Table 2.** Effect of curcumin on dopamine, DOPAC, and HVA levels (µg/g) in the striatum 22 days after 6-OHDA exposure.

| Group | Dopamine | DOPAC | HVA |
|---|---|---|---|
| Sham-vehicle | 7.18 ± 0.23 | 7.079 ± 0.6 | 0.44 ± 0.02 |
| Sham-curcumin | 8.68 ± 0.58$^{\#}$ | 7.085 ± 0.54 | 0.44 ± 0.02 |
| 6-OHDA-vehicle | 2.75 ± 0.51$^{\#\#\#}$ | 3.05 ± 0.67$^{\#\#\#}$ | 0.26 ± 0.02$^{\#\#\#}$ |
| 6-OHDA-curcumin | 4.90 ± 0.68 $^{**,\#\#}$ | 4.67 ± 0.49$^{\#}$ | 0.33 ± 0.03$^{\#}$ |

The data are expressed as mean ± SEM ($n$ = 10-12/group). $^{**}p < 0.01$, compared with 6-OHDA-vehicle group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, compared with sham-vehicle group (ANOVA followed by Newman-Keuls test).

**Table 3.** Effect of curcumin on 5-HT, 5-HIAA, NE, and DHPG levels (µg/g) in the hippocampus 22 days after 6-OHDA exposure.

| Group | 5-HT | 5-HIAA | NE | DHPG |
|---|---|---|---|---|
| Sham-vehicle | 0.39 ± 0.04 | 0.89 ± 0.03 | 0.54 ± 0.04 | 0.88 ± 0.06 |
| Sham-curcumin | 0.32 ± 0.03 | 0.81 ± 0.05 | 0.58 ± 0.04 | 0.85 ± 0.06 |
| 6-OHDA-vehicle | 0.34 ± 0.04 | 0.83 ± 0.04 | 0.25 ± 0.05$^{###}$ | 0.51 ± 0.09$^{##}$ |
| 6-OHDA-curcumin | 0.33 ± 0.05 | 0.85 ± 0.10 | 0.31 ± 0.05$^{##}$ | 0.80 ± 0.08***** |

The data are expressed as mean ± SEM ($n$ = 10-12/group). *$p$ < 0.05, compared with 6-OHDA-vehicle group; $^{##}p$ < 0.01, $^{###}p$ < 0.001, compared with sham-vehicle group (ANOVA followed by Newman-Keuls test).

*Immunohistochemistry*

As shown in Fig. 4, the sham groups exhibited a strong immunoreactivity of TH-immunoreactive neurons in the SNpc. The 6-OHDA-vehicle group exhibited a reduction of the expression of these neurons. The density of TH-immunoreactive neurons in the 6-OHDA-vehicle group, calculated by comparing the optical density of each image, significantly differed from the sham-vehicle group ($p$ < 0.01) and sham-curcumin group ($p$ < 0.001; $F_{3,83}$ = 8.903, $p$ < 0.0001). Fig. 4 also shows that the 6-OHDA-curcumin group exhibited a slight reduction of TH-positive neurons. Moreover, the 6-OHDA-vehicle group exhibited a significant decrease in TH-immunoreactive neurons in the SNpc compared with the 6-OHDA-curcumin group ($p$ < 0.05).

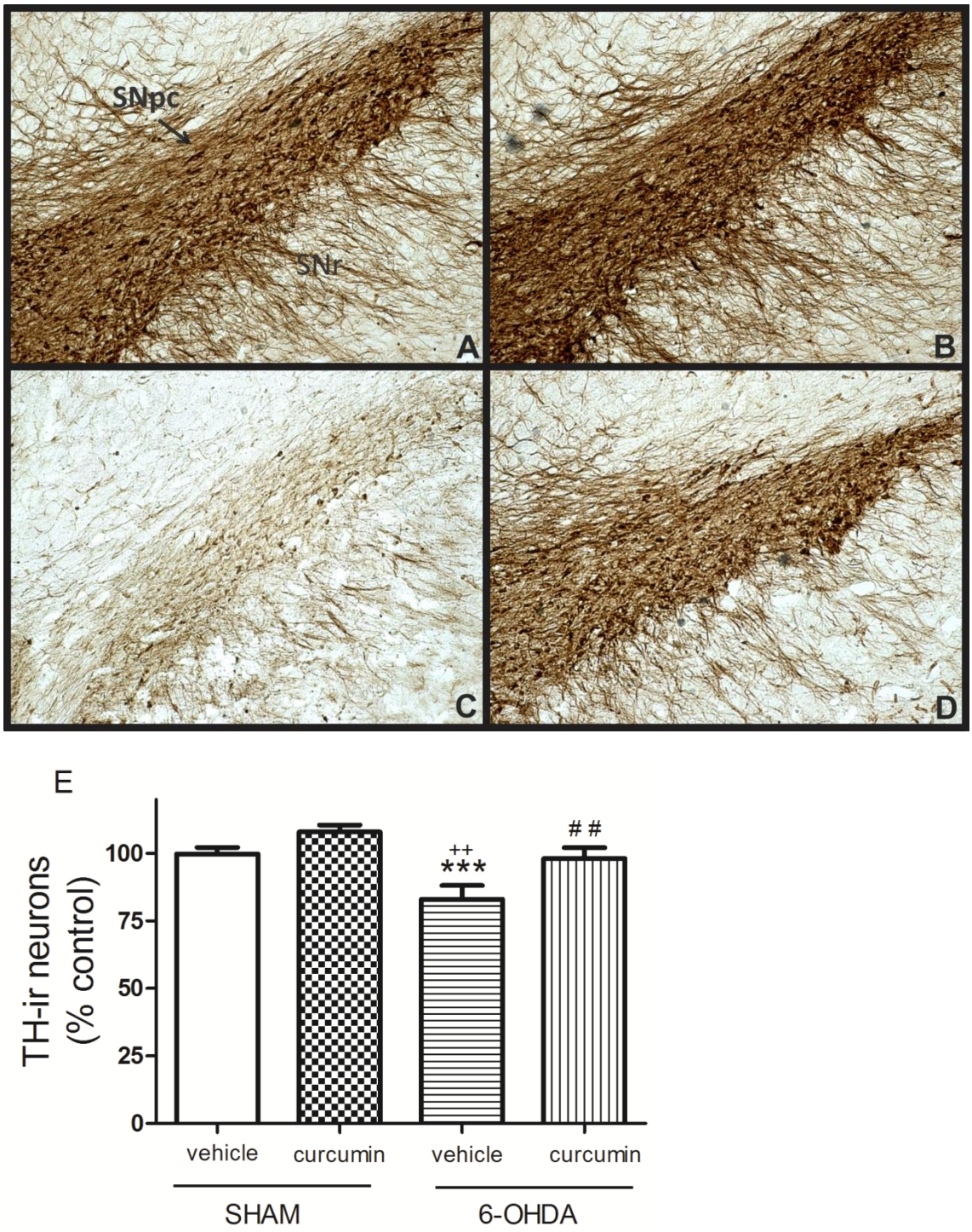


**Figure 4.** Effects of curcumin on TH-immunoreactive neurons in the SNpc. Immunohistochemistry figures show the density of TH-immunoreactive neurons in the SNpc in all of the groups 22 days after 6-OHDA infusion. (A) Sham-vehicle. (B) Sham-curcumin. (C) 6-OHDA-vehicle. (D) 6-OHDA-curcumin. (E) Percentage of TH-immunoreactive neurons, determined by calculating the optical density. The data are expressed as mean ± SEM ($n$ = 3-4/group). $^{\#\#}p <$ 0.01, compared with 6-OHDA-vehicle group; $^{++}p < 0.01$, compared with sham-vehicle group; $^{***}p < 0.001$, compared with sham-curcumin group (ANOVA followed by Newman-Keuls test).

## Discussion

Dopaminergic neuron death in the SNpc induced by injection of 6-OHDA resulted in depressive-like behavior and an anhedonic-like state in rats. Curcumin treatment (30 mg/kg, p.o.) for 21 days exerted a neuroprotective effect against 6-OHDA, decreasing depressive-like behavior and the anhedonic-like state and increasing monoamines levels.

To discard the possible influence of baseline locomotor activity before testing the animals in behavioral models of depression, we performed the open field test. Hypolocomotion observed 24 h after surgery in rats that received 6-OHDA was not reversed by a single dose of curcumin. The decrease in motor behavior is likely attributable to neuronal loss observed 24 h after neurotoxin exposure. This reduction of locomotor activity was reversed in both lesioned groups on day 21, ensuring that motor influences did not affect the results obtained in the behavior models of depression.

To evaluate the antidepressant-like effect of curcumin, the forced swim test and sucrose preference test were performed to determine whether curcumin can reverse the depressive-like effects of the neurotoxin. Sucrose preference in the 6-OHDA-vehicle group was lower than in the control groups, indicating an anhedonic-like state caused by the neurotoxin in these rats. These results are consistent with previous studies, in which the neurotoxin provoked dopaminergic neuron damage and led to an anhedonic-like state (Li et al., 2009; Santiago et al., 2010). Rats treated with curcumin that received 6-OHDA did not exhibit a reduction of sucrose preference compared with control rats. Moreover, we observed an increase in sucrose preference in these animals on day 14 compared with the 6-OHDA-vehicle group. These data indicate that curcumin

can reverse the anhedonic-like state induced by 6-OHDA. Previous studies that used other models of depressive-like behavior demonstrated curcumin's ability to reverse anhedonic-like states induced by chronic unpredictable mild stress and chronic corticosterone administration (Li et al., 2009; Huang et al., 2011; Lopresti et al, 2012). The present study further shows a similar effect of curcumin in the 6-OHDA-induced model of anhedonia.

In the forced swim test, an increase in immobility time is related to depressive-like behavior. Bilateral infusion of 6-OHDA in the SNpc, similar to a previous study (Santiago et al., 2010), increased immobility time and reduced swimming time. In contrast, the 6-OHDA-curcumin group exhibited a reduction of immobility time and increase in swimming parameters compared with the 6-OHDA-vehicle group. Interestingly, in sham animals, the dose of curcumin used did not exert antidepressant-like activity in the forced swim test, in which no significant difference was observed in immobility time between the sham-vehicle and sham-curcumin groups. This result is consistent with the lack of changes in monoamine content observed in the hippocampus. These results indicate that the effect of curcumin in 6-OHDA-lesioned rats was not attributable to nonspecific effects. However, a significant effect on swimming time was found. This may have been caused by a threshold effect of the curcumin dose used (i.e., although it increased swimming behavior, this was not sufficient to decrease immobility).

To corroborate the behavioral test results, neurochemical and immunohistochemistry analyses were performed. In the analysis of the hippocampus, NE concentrations were more affected by the neurotoxin than 5-HT concentrations. The 6-OHDA-vehicle group exhibited a decrease in NE and

its metabolite DHPG compared with controls. The neurotoxin group treated with curcumin exhibited a decrease in NE levels compared with controls. However, DHPG levels were significantly higher in the 6-OHDA-curcumin group than in the 6-OHDA-vehicle group and did not significantly differ from the sham groups. The concentrations of 5-HT and its metabolite in the hippocampus were statistically equal among groups. In other animal models of depression, such as chronic unpredictable mild stress and olfactory bulbectomy (Xu et al., 2005b; Li et al., 2009), curcumin altered serotoninergic function and concentration. In the present 6-OHDA model, curcumin did not alter 5-HT levels in the hippocampus. The curcumin groups did not exhibit an increase in 5-HT concentrations, but the 6-OHDA-vehicle group also did not exhibit a decrease in 5-HT concentrations. With regard to striatal DA, the 6-OHDA-vehicle group exhibited a large decrease in the levels of this neurotransmitter compared with controls. This decrease was also apparent when we measured the DA metabolites DOPAC and HVA, indicating the death of dopaminergic neurons. The same conclusion may be drawn from the immunohistochemical analyses, in which the 6-OHDA-vehicle group exhibited minor expression of TH-immunoreactive neurons. The 6-OHDA-curcumin group exhibited an increase in DA concentrations compared with the 6-OHDA-vehicle group, also supporting the histological analyses and suggesting a neuroprotective effect of curcumin. DOPAC and HVA levels in the 6-OHDA-curcumin group were also higher, coinciding with the DA concentrations, but this increase did not significantly differ from the 6-OHDA-vehicle group.

Curcumin may be considered a multi-target drug that exerts its neuroprotective and antidepressant-like effects in the 6-OHDA model through

numerous mechanisms (Hurley and Tizabi, 2013). Neuronal death induced by 6-OHDA might be prevented through antiinflammatory and antioxidant mechanisms (Sandur et al., 2007; Jurenka, 2009; Wang et al., 2009). Curcumin can exert protective effects by inhibiting glial activation and reducing the expression of pro-apoptotic proteins, such as nuclear factor-κB and caspase-3 (Wang et al., 2009; Arora, 2011; Tripanichkul and Jaroensuppaperch, 2012). Although the antidepressant-like effect of curcumin appears to be mainly related to alterations in serotoninergic function in animal models of depression (Wang et al., 2008), its antidepressant-like effect in the 6-OHDA-induced model of PD may be modulated by a dopaminergic mechanism. Previous studies have correlated curcumin treatment with an increase in the levels of DA and its metabolites in both PD and depression models (Xu et al., 2005a; Xu et al, 2005b; Kulkarni et al., 2008; Agrawal et al., 2012; Du et al., 2012; Pan et al.,2012;Tripanichkul and Jaroensuppaperch, 2012;Hurley and Tizabi, 2013). Imaging studies found a strong correlation between impairment of the DA system and depression symptoms in PD patients (Brooks and Piccini, 2006; Rektorova et al., 2008). Moreover, PD-related depression appears to be correlated with dopaminergic pathways. Studies of 5-HT in PD have provided inconclusive results. Patients with PD present with a reduction of DA pathway activity associated with reward and mood systems. These data support the hypothesis that the dopaminergic system is mainly involved in the etiology of PD-related depression (Remy et al., 2005; Aarsland et al., 2012).

Altogether, the present results confirmed our hypothesis that curcumin exerts antidepressant-like effects in PD-related depression. Using two behavior models of depression, curcumin treatment reversed the depressive-like effect of

6-OHDA, confirming the antidepressant-like effect of curcumin in this model. The behavioral results were corroborated by the neurochemical and immunohistological analyses. The present study indicates that the antidepressant-like effect of curcumin in this model is more related to the preservation of dopaminergic function compared with other monoamines.

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**Conflict of interest**

The authors have no conflict of interest to declare.

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## 4. CONCLUSÕES

- A 6-OHDA foi capaz de induzir comportamento tipo-depressivo e estado anedônico em ratos, verificados pelos TNFm e teste de preferência pela sacarose;
- O tratamento prolongado com a curcumina (30mg/kg, p.o.) protegeu contra o desenvolvimento de comportamento tipo-depressivo e estado anedônico induzidos pela infusão intranigral de 6-OHDA;
- A administração de uma dose de curcumina, 1 hora após a cirurgia estereotáxica, não reverteu o comprometimento motor dos animais causado pela infusão da 6-OHDA;
- A infusão intranigral da 6-OHDA promoveu redução na concentração de DA, DOPAC e HVA no estriado e NA e DHPG no hipocampo, bem como uma redução na densidade de neurônios TH-imunoreativos na SNpc;
- O estriado e a SNpc dos animais tratados com curcumina apresentaram uma maior concentração de DA e uma maior densidade de neurônios TH-imunoreativos, respectivamente, quando comparados ao grupo 6-OHDA-veículo.

Tomados em conjunto, nossos resultados indicam que a curcumina apresentou efeito tipo-antidepressivo e neuroprotetor no modelo de parkinsonismo induzido por 6-OHDA. Além disso, os dados apontam que estes efeitos parecem estar mais relacionados à preservação das funções dopaminérgicas.

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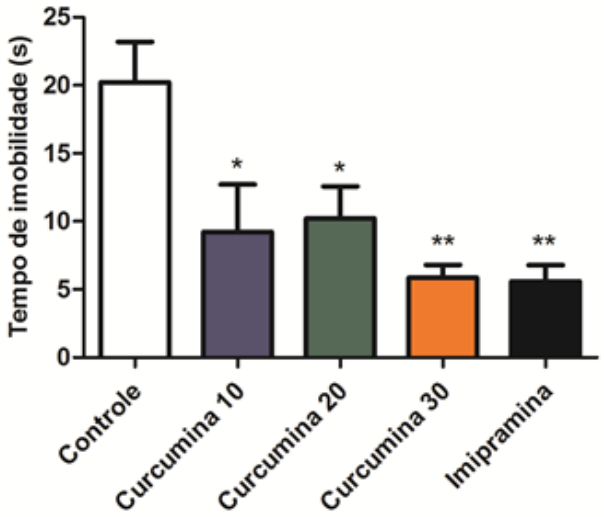
## 6. ANEXO – Curva dose-resposta

Investigação do possível efeito tipo-antidepressivo de diferentes doses de curcumina (10, 20 e 30mg/kg, p.o) administradas 24, 8 e 1 h antes do teste de TNFm.




Avaliação do comportamento tipo-depressivo dos animais tratados com diferentes doses de curcumina no TNFm. Valores expressos como média ± erro padrão da média (n=9/grupo). *p<0,05, **p<0,01 comparados com o grupo controle. ANOVA seguida de post hoc de Newman-Keuls.