UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
CURSO DE GRADUAÇÃO EM BIOMEDICINA

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COMPARAÇÃO DA ATIVIDADE ANTIOXIDANTE *IN VITRO* DE DIFERENTES PREPARAÇÕES DE CURCUMINA

Porto Alegre
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Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharela em Biomedicina.

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Dados Internacionais de Catalogação na Publicação

Panta Rodrigues da Silva, Giovana
Comparação da atividade antioxidante in vitro de diferentes preparações de curcumina / Giovana Panta Rodrigues da Silva. -- 2019.
54 f.
Orientador: Angelo Piato.

Coorientadora: Adrieli Sachett.

Trabalho de conclusão de curso (Graduação) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Curso de Biomedicina, Porto Alegre, BR-RS, 2019.

1. Estresse oxidativo. 2. Antioxidantes. 3. Curcumina micronizada. I. Piato, Angelo, orient. II. Sachett, Adrieli, coorient. III. Título.

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Aprovado em: 03 de julho de 2019.

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AGRADECIMENTOS

Primeiramente agradeço a Deus, por sempre guiar, iluminar e proteger meu caminho, o qual me deu força e paciência para continuar nessa caminhada e alcançar meu objetivo.

Agradeço à minha família, por sempre estarem ao meu lado, por terem confiado e acreditado no meu potencial principalmente nos meus momentos de incertezas.

Ao meu amigo Alexandre, que mesmo reclamando de quase tudo, sempre esteve disposto a ajudar no que fosse preciso durante toda essa caminhada, agradeço também por cada chimarrão e momento pós-prova.

Aos meus amigos Marilia, Guilherme, Murilo e Marcus, com os quais vivi os momentos mais maravilhosos, que me fizeram esquecer totalmente da pressão e dos problemas que surgiam em minha vida. E em especial à Larissa que, além disso, sempre esteve disposta a ouvir aos meus desabafos. Obrigada pela parceria de sempre!

Às minhas amigas Mônia, Franciele e Letícia pelos resumos compartilhados e por tantos momentos de alegria. Aos meus amigos Eloisa, Maia, Lucas e Raul pelas conversas e distrações.

Ao grupo LAPCOM, que nunca mediram esforços para transmitir seus conhecimentos, bem como a esclarecer qualquer dúvida que surgia.

À minha coorientadora Adrieli, a quem admiro por sua determinação e perseverança, obrigada pela paciência e por cada ensinamento compartilhado.

Ao meu orientador Angelo, que desde o início esteve com as portas do laboratório abertas e por ter me acolhido de imediato. Também agradeço ao vasto aprendizado passado de forma clara e precisa. Muito obrigada!

“A educação é a arma mais poderosa que
você pode usar para mudar o mundo.”
(Nelson Mandela)

RESUMO

Espécies reativas de oxigênio e os radicais livres são produzidos durante o metabolismo aeróbico normal. A superprodução dessas espécies está relacionada com o estresse oxidativo que pode levar a oxidação de proteínas, lipídios, DNA e desencadear uma série de eventos que resultam na morte celular. O estresse oxidativo também está relacionado à fisiopatologia de doenças neurodegenerativas e transtornos mentais como ansiedade e depressão. Assim, antioxidantes podem potencialmente ser utilizados como tratamento e/ou adjuvantes nessas condições. Nesse contexto, a curcumina, um polifenol extraído do rizoma de *Curcuma longa L* (Zingiberaceae) possui atividades antioxidante e anti-inflamatória, entretanto, apresenta baixa biodisponibilidade. Dessa forma, o objetivo desse trabalho foi comparar a atividade antioxidante *in vitro* da curcumina (CUR, 0,0625, 0,25 e 1 g/L) com uma preparação de curcumina micronizada (CM, 0,0625, 0,25 e 1 g/L) sobre as capacidades redutora de ferro (FRAP) e removedora de radicais livres (DPPH), bem como sobre o efeito protetor contra a oxidação da glutationa (GSH) induzida pelo peróxido de hidrogênio e inibição da formação de radical hidroxila. Ácido ascórbico (0,0625, 0,25 e 1 g/L) foi utilizado como controle positivo. A CM demonstrou maior capacidade removedora de radical livre do que a CUR no ensaio de DPPH nas concentrações de 0,0625 e 1 g/L e equivalente ao do ácido ascórbico em todas as concentrações. CM apresentou maior potencial redutor que CUR nas concentrações de 0,0625 e 1 g/L, sendo ambas menos efetivas que o ácido ascórbico. Nenhum dos grupos apresentou diferença significativa sobre o efeito protetor contra oxidação da GSH. Porém, sobre a inibição da formação de radical hidroxila a CM e CUR foram significativamente mais eficazes que o ácido ascórbico na concentração de 1 g/L. Os resultados mostram pela primeira vez que o potencial antioxidante da CM é superior ao da CUR. Mais estudos são necessários para a elucidação do mecanismo antioxidante da CM e de seus potenciais efeitos *in vivo*.

Palavras-chave: estresse oxidativo; antioxidante; curcumina micronizada.

ABSTRACT

Reactive oxygen species (ROS) and free radicals are produced during normal aerobic metabolism. The overproduction of ROS is related to oxidative stress and lead to oxidation of proteins, lipids, DNA, and trigger a series of events that result in cell death. Oxidative stress is also related to the pathophysiology of neurodegenerative diseases and mental disorders such as anxiety and depression. Thus, antioxidants can potentially be used as treatment and/or adjuvants under such conditions. In this context, curcumin, a polyphenol extracted from the rhizome of Curcuma longa L (Zingiberaceae) has antioxidant and anti-inflammatory activities, however, presents low bioavailability. The aim of this study was to compare the *in vitro* antioxidant activity of curcumin (CUR, 0.0625, 0.25 and 1 g/L) with a micronized curcumin preparation (CM, 0.0625, 0.25 and 1 g/L) on the iron-reducing (FRAP) and free radical scavenging (DPPH) capacities, as well as on the protective effect against hydrogen peroxide-induced glutathione (GSH) oxidation and inhibition of hydroxyl radical formation. Ascorbic acid (0.0625, 0.25 and 1 g/L) was used as a positive control. The CM showed higher free radical scavenging capacity than the CUR in the DPPH assay at concentrations of 0.0625 and 1 g/L and equivalent to the ascorbic acid at all concentrations. CM presented higher reduction potential than CUR at concentrations of 0.0625 and 1 g/L, and both were less effective than ascorbic acid. None of the groups presented a significant difference in the protective effect against oxidation of GSH. However, on the inhibition of hydroxyl radical formation, CM and CUR were significantly more effective than ascorbic acid at the concentration of 1 g/L. The results show for the first time that the antioxidant potential of CM is superior to CUR. Further studies are needed to elucidate the antioxidant mechanism of CM and its potential *in vivo* effects.

Keywords: oxidative stress; antioxidant; micronized curcumin.

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1 INTRODUÇÃO

O sistema nervoso central demanda grande quantidade energética e consequentemente elevada taxa metabólica , representando 20% do consumo total de oxigênio do organismo (FEDOCE et al., 2018; HALLIWELL; GUTTERIDGE, 1985). Um elevado consumo de oxigênio está relacionado com maior produção de espécies reativas de oxigênio (ERO's) e isso potencialmente pode causar danos às células do sistema nervoso central (ŞAHIN; GÜMÜŞLÜ, 2007).

ERO's podem ser classificadas em radicais livres (superóxido, $O_2^{\cdot -}$), radical hidroxila ($OH^{\cdot}$), ou não radicais, como peróxido de hidrogênio (H_2O_2) (Figura 1) (GANDHI; ABRAMOV, 2012). São geradas no organismo por fontes exógenas (como as radiações ultravioleta e ionizante e fármacos) e endógenas (resultado do processo de respiração celular). Na mitocôndria, as ERO's são formadas pela cadeia respiratória de elétrons através de cinco complexos: NADH-coenzima Q redutase (CoQ, complexo I), succinato desidrogenase (complexo II), coenzima Q-citocromo c redutase (complexo III), citocromo c oxidase (complexo IV) e ATP sintase (complexo V) (Figura 2) (MAAS; VALLÈS; MARTENS, 2017). Sendo os complexos I e III os principais responsáveis pela produção de O_2^- mitocondrial (ORELLANA; SLACHEVSKY, 2013; ZUCKERMAN et al., 2003).

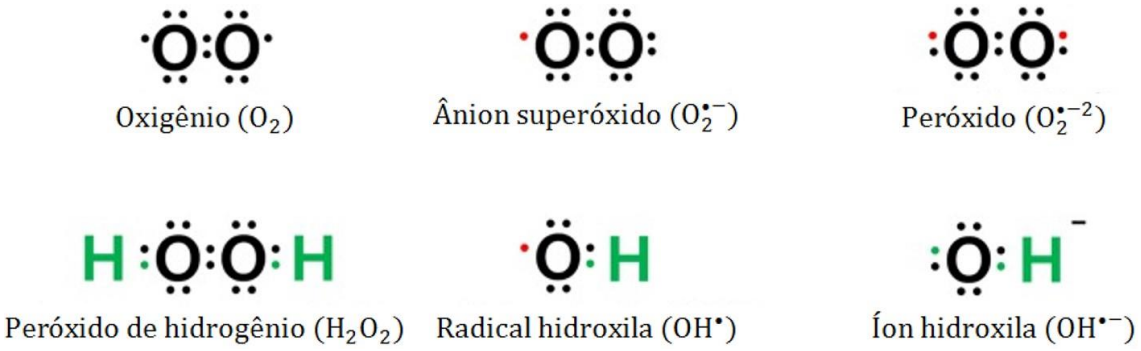

Figura 1. Exemplos de espécies reativas de oxigênio (ERO's). A redução consecutiva de oxigênio através da adição de elétrons causa a formação de uma variedade de ERO's que incluem ânion superóxido ($O_2^{\cdot -}$), radical hidroxila ($OH^{\cdot}$), íon hidroxila ($OH^{\cdot}$) e peróxido de hidrogênio (H_2O_2). O ponto vermelho indica um elétron desemparelhado. Fonte: adaptado de KIM et al., (2015).

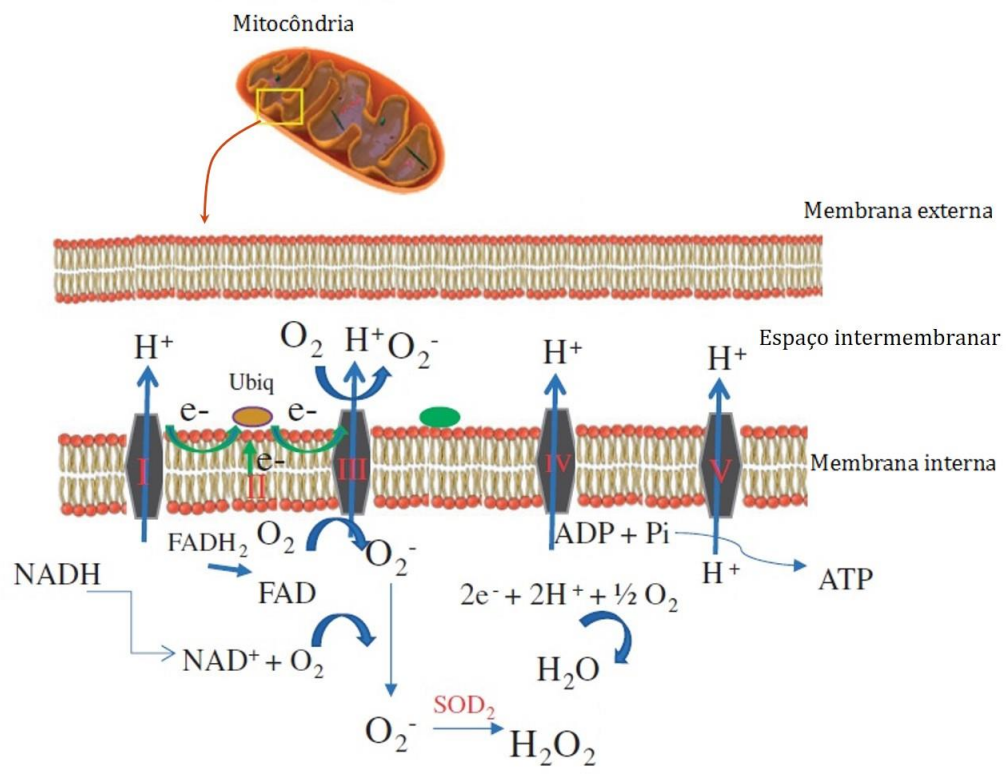

Figura 2. Formação de espécies reativas de oxigênio na cadeia transportadora de elétrons (CTE). No ciclo de Krebs ocorre uma série de reações enzimáticas que fornecem elétrons (do piruvato via acetil-CoA) à CTE na forma de NADH e $FADH_2$. Esses elétrons passam então por transporte vetorial ao longo da CTE, gerando um gradiente de energia eletroquímica pelo qual o adenosina difosfato (ADP) pode ser fosforilado em adenosina trifosfato (ATP) no complexo V. Para manter o fluxo de elétrons (e geração de ATP) os elétrons devem ser "removidos" da CTE, e isso é realizado no complexo IV (citocromo-oxidase), onde os elétrons reduzem o oxigênio à água em quatro etapas consecutivas (mas combinadas) de um elétron. Embora todo o processo seja realmente eficiente, cerca de 1-2% do oxigênio molecular consumido durante a respiração fisiológica normal é reduzido em reações colaterais de um elétron (principalmente no complexo I e III) no radical ânion superóxido, $O_2^{\cdot-}$ (também comumente apenas chamado "superóxido"). O superóxido assim gerado é quase imediatamente dismutado em peróxido de hidrogênio (H_2O_2) pela superóxido dismutase (SOD). Fonte: adaptado de Fedoce et al, 2018.

Em homeostase, a produção de ERO's é controlada por diversos sistemas antioxidantes enzimáticos e não enzimáticos (SENKOWSKI; GALLINAT, 2015). A enzima superóxido dismutase (SOD) catalisa a dismutação do $O_2^{\cdot-}$ a H_2O_2, utilizando ferro ou manganês como cofator (DASURI; ZHANG; KELLER, 2013; GANDHI; ABRAMOV, 2012), e então, o H_2O_2 é eliminado pela enzima catalase (CAT) no citosol ou pela glutationa peroxidase (GPx) na mitocôndria (FEDOCE et al., 2018). A glutationa (GSH) é sintetizada a partir do dipeptídeo γ em combinação com a glicina pela ação da glutationa sintetase, consistindo em três aminoácidos, glutamato, cisteína e glicina. Sendo o grupo tiol da cisteína o local ativo e responsável por suas funções protetoras contra o estresse oxidativo e que reconhecido como

o tiol não proteico mais importante nos sistemas vivos (DASURI; ZHANG; KELLER, 2013; GANDHI; ABRAMOV, 2012). A GSH reduzida está envolvida na remoção de ERO's, como $O_2^{\cdot -}$ e $OH^{\cdot}$, além de doar elétrons para redução de peróxidos pela GPx através da reação de oxidação da GSH em glutationa oxidada na forma dimerizada (GSSG) (Figura 3). A GSH também pode sofrer oxidação e formar dissulfetos do tipo GSSR com o tiol da cisteína presente em proteínas.

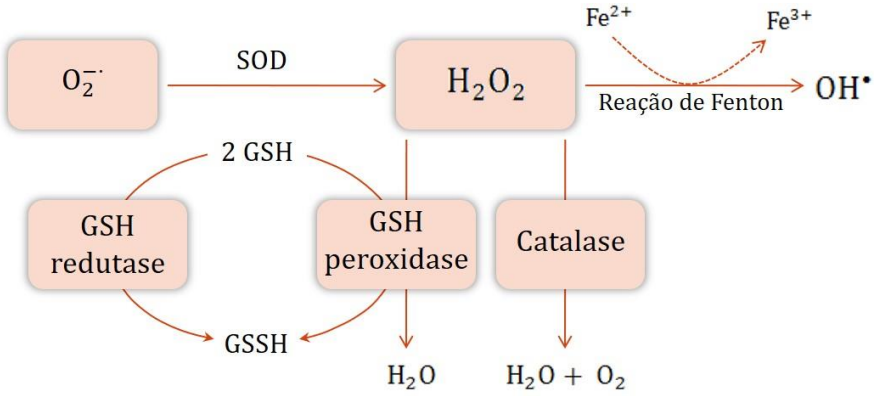

Figura 3. Enzimas envolvidas na geração e inativação de espécies reativas de oxigênio. O H_2O_2 pode sofrer conversão espontânea para o radical hidroxila ($OH^{\cdot}$) por meio da reação de Fenton. O $OH^{\cdot}$ é extremamente reativo e ataca a maioria dos componentes celulares. O H_2O_2 pode ser desintoxicado via glutationa (GSH) peroxidase ou catalase para H_2O e O_2. Fonte: adaptado de Li et al, 2013.

Quando ocorre uma superprodução de ERO's e uma diminuição nas defesas antioxidantes, há geração de $OH^{\cdot}$ via reação de Fenton dependente de íons metálicos. Isso resulta em estresse oxidativo que altera a homeostase neuronal e favorece a ocorrência de lesões oxidativas em proteínas, lipídios e ácidos nucléicos, podendo resultar em morte celular (DASURI; ZHANG; KELLER, 2013; GANDHI; ABRAMOV, 2012; MAAS; VALLÈS; MARTENS, 2017; UTTARA et al., 2009; ZHANG et al., 2013). Esse desequilíbrio parece estar relacionado à fisiopatologia de diversas condições centrais como doenças neurodegenerativas, transtornos mentais como ansiedade e depressão e também com a hipertensão arterial pulmonar e aterosclerose (BUTTERFIELD; HALLIWELL, 2019; GILHOTRA; DHINGRA, 2010; KANCHANATAWAN et al., 2018; MACCARTHY; SHAH, 2003; MANOHARAN et al., 2016; SAMARGHANDIAN et al., 2017).

Quando um organismo é submetido a uma determinada situação estressora, diversos sistemas, como o sistema nervoso autônomo e eixos neuroendócrinos, como o eixo hipotálamo-hipófise-adrenal (HPA) são ativados a fim de responder adequadamente a

essa demanda (MCEWEN, 2006). Tal resposta (chamada de alostase) tem por objetivo superar as demandas e, posteriormente, a homeostase é reestabelecida. Entretanto, em determinados casos (como em situações de estresse crônico), o restabelecimento da homeostasia pode ser prejudicado, predispondo o indivíduo a efeitos deletérios que resultam no estabelecimento de um fenômeno chamado de sobrecarga alostática (FEDOCE et al., 2018; RASGON; MCEWEN, 2016). Esse fenômeno está relacionado à hiperativação do eixo HPA, com aumento dos níveis de cortisol, que por sua vez está relacionados a um aumento de dano oxidativo (FEDOCE et al., 2018).

Além da produção excessiva de ERO's e da hiperativação do eixo HPA, a neuroinflamação parece estar presente em diversos transtornos neuropsiquiátricos (SKAPER; FACCI; GIUSTI, 2014; STREIT, 2010; SVENUNGSSON et al., 2001). Diversos estudos já demonstraram que em pacientes com transtornos mentais como ansiedade e depressão, os níveis de citocinas pró-inflamatórias como fator de necrose tumoral α (TNF-α), interleucinas 1β e 6 (IL-6) e interferon alfa (INF-α) estão aumentados (CRASKE et al., 2017; FEDOCE et al., 2018; GILHOTRA; DHINGRA, 2010; HEWLINGS; KALMAN, 2017; KANCHANATAWAN et al., 2018). Esse "ambiente inflamado" pode levar a um aumento na produção de ERO's ou vice-versa (FEDOCE et al., 2018).

Apesar de não ser claro o que se estabelece antes, se é o estresse oxidativo/neuroinflamação ou os transtornos neuropsiquiátricos, vislumbra-se que compostos antioxidantes, capazes de contrabalancear a geração de espécies reativas nas células e restabelecer a homeostase, possam ser utilizados como uma terapia adjunta para tais condições.

A curcumina é o principal polifenol natural encontrado no rizoma de *Curcuma longa* L. (Zingiberaceae) nativa da Ásia (figura 4). Os principais constituintes ativos da curcumina são os curcuminóides e os sesquiterpenos, os quais são responsáveis por aumentar a solubilidade das derivações de curcumina e assim, adequam à formulação de fármacos. Os curcuminóides são fenóis naturais e comumente utilizados como corantes ou aditivos alimentares. A composição química desta molécula confere menos solubilidade em água com pH ácido e neutro, mas é altamente solúvel em metanol ou etanol. Os grupos aromáticos da curcumina oferecem hidrofobicidade à molécula e o ligante confere flexibilidade, mas também, consiste em uma molécula lipofílica, capaz de atravessar a barreira hematoencefálica, não apresentando efeitos tóxicos em animais

e em humanos mesmo em elevadas doses (AMALRAJ et al., 2017; HATCHER et al., 2008; XU et al., 2005).

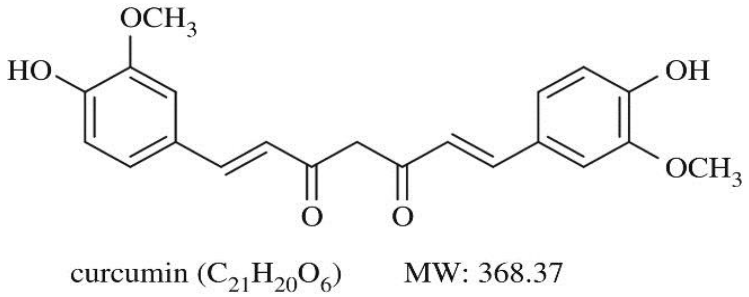

Figura 4. Estrutura química da curcumina extraída do rizoma de *Curmuma longa*. Nomenclatura IUPAC: (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione (PUBCHEM, [s.d.]).

Vários estudos demonstram o efeito antioxidante da curcumina. Tal efeito pode seratribuído aos grupos fenólicos presentes na molécula (ZHENG et al., 2017). Esses grupos possuem potencial antioxidante devido às suas propriedades como agentes redutores, doadores de hidrogênio, assim como inibidores de adsorção de oxigênio. Além disso, acredita-se que seja necessária a formação de radical fenóxi estável, pois o grupo fenólico parece não ser, por si só, capaz de eliminar os radicais livres dos curcuminóides (BUTTERFIELD; HALLIWELL, 2019; SAHU, 2016). Estudos demonstram que assim como a fluoxetina, a curcumina foi capaz de restaurar o comportamento e as mudanças neuroquímicas associadas à ansiedade. Através de seu papel na modulação da neuroinflamação a partir da inibição do fator nuclear NF-κB, responsável pela regulação da ativação de citocinas inflamatórias, como o interferon alfa (INF-α) (HATCHER et al., 2008). Além disso, a curcumina melhora a cognição, assim como a neurogênese através de mecanismo que envolve o aumento dos níveis do fator neurotrófico derivado do cérebro (BDNF) (ZHANG et al., 2015). Por outro lado, estudos mostram que a curcumina também apresenta um efeito tipo-ansiolítico devido ao aumento nos níveis de serotonina no hipocampo e córtex pré-frontal, além de atuar modulando o sistema GABAérgico (BENAMMI et al., 2014; GILHOTRA; DHINGRA, 2010).

Embora a curcumina apresente diversos efeitos benéficos, estudos demonstraram que a biodisponibilidade é baixa (ANAND et al., 2007). Uma alternativa para melhorar a biodisponibilidade e potencializar os seus efeitos consiste na técnica de micronização da matéria-prima através da técnica de dióxido de carbono supercrítico (AGUIAR et al., 2017; FRANCESCHI et al., 2008). A qual permite a obtenção de um tamanho bem

reduzido da partícula. E dessa maneira, há um aumento da solubilidade do composto, devido ao aumento da área de superfície de contato e uma melhora na biodisponibilidade de compostos pouco solúveis em água (BERTONCELLO et al., 2018; BHAKAY et al., 2011). Essa abordagem vem sendo utilizada em diversos estudo que confirmam a melhoria de moléculas. A micronização de N-acetilcisteína resultou em um aumento na taxa de dissolução do composto, assim como uma modificação da estrutura cristalina (AGUIAR et al., 2017). A micronização do resveratrol aumentou sua biodisponibilidade em pacientes com câncer colorretal e reduziu os efeitos adversos em indivíduos saudáveis (HOWELLS et al., 2011). Além disso, desmonstrou-se que a curcumina micronizada previne crises epilépticas de uma maneira mais robusta do que a forma convencional (BERTONCELLO et al., 2018).

Portanto, considerando que o processo de micronização é capaz de aumentar a superfície de contato, taxa de dissolução e solubilidade do composto. Nossa hipótese é que a CM apresente capacidade antioxidante superior à CUR.

2 JUSTIFICATIVA

A neurobiologia dos transtornos mentais é complexa e há presença de desequilíbrio entre a produção de espécies reativas dos sistemas antioxidantes. A investigação de compostos antioxidantes com foco na oxidação celular pode fornecer candidatos a novas terapias para tais condições. A curcumina apresenta baixa biodisponibilidade e isso pode limitar o seu uso na clínica. A micronização é um processo capaz de melhorar significativamente a cinética de moléculas. Nesse sentido, é mandatório comparar os diferentes tipos de preparações de curcumina a fim de fornecer evidências para os estudos *in vivo* subsequentes.

3 OBJETIVOS

3.1 Objetivo geral

Comparar a atividade antioxidante da curcumina (CUR) e da curcumina micronizada (CM) *in vitro.*

3.2 Objetivos específicos

Comparar a atividade antioxidante das duas preparações sobre os seguintes parâmetros: (a) eliminação do radical DPPH; (b) poder antioxidante redutor de ferro (FRAP); (c) proteção contra a oxidação da glutationa (GSH) e (d) inibição da formação de radical hidroxila (desoxirribose).

4 ARTIGO CIENTÍFICO

Comparação da atividade antioxidante *in vitro* de diferentes preparações de curcumina

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RESUMO

Curcumina é um polifenol extraído do rizoma de *Curcuma longa L* (Zingiberaceae). Esse composto apresenta atividade antioxidante, anti-inflamatória, ansiolítica, antidepressiva e neurotrófica já demonstradas em modelos pré-clínicos e clínicos. Entretanto, pelas suas características físico-químicas apresenta baixa biodisponibilidade. A micronização por fluido supercrítico é um processo que permite reduzir a granulometria de algumas partículas químicas e, dessa forma, alterar aspectos físico-químicos da molécula melhorando por exemplo a sua biodisponibilidade. Nesse contexto, o objetivo do trabalho foi comparar a atividade antioxidante *in vitro* da curcumina (CUR), curcumina micronizada (CM) e ácido ascórbico nas concentrações de 0,0625, 0,25 e 1 g/L. CM demonstrou maior capacidade removedora de radicais livres e potencial redutor do que a CUR, sendo equivalente ao do ácido ascórbico. Todos os compostos apresentaram efeito protetor contra oxidação da glutationa e foram capazes de inibir a formação do radical hidroxila. Nossos resultados demonstram pela primeira vez que CM possuí maior capacidade antioxidante que a CUR.

Palavras-chave: estresse oxidativo; antioxidante; curcumina micronizada

1. INTRODUÇÃO

A curcumina é um polifenol derivado do rizoma de *Curcuma longa L.* (Zingiberaceae), amplamente utilizada tradicionalmente como tempero, conservante de alimentos e erva medicinal devido a suas diversas propriedades antioxidante, anti-inflamatória, antimicrobiana e antitumoral em diversos países do sudeste asiático e ao redor do mundo (LESTARI; INDRAYANTO, 2014; REDDY et al., 2005; SA; DAS, 2008; WRIGHT et al., 2013). Sua estrutura é composta por dois grupos fenóis metoxilados, um grupo β-dicetona e isômeros ceto e enol apresentando insolubilidade em água (Barzegar, 2012).

Diversos estudos têm demonstrado os efeitos benéficos da curcumina. Atua como antioxidante através do aumento na eliminação de ERO's (Menon & Sudheer, 2007), da inibição de enzimas geradoras de ERO's, como a lipoxigenase e do aumento na atividade de enzimas antioxidantes, como a superóxido dismutase (Lin et al., 2007;

Panahi, Alishiri, Parvin, & Sahebkar, 2016). Ao ser administrada oralmente a curcumina foi capaz de aumentar os níveis de serotonina e noradrenalina tanto no córtex pré-frontal quanto no hipocampo em modelo de depressão em camundongos (Xu et al., 2005) e melhorou o quadro depressivo e a ansiedade em humanos em um estudo randomizado e controlado (Lopresti & Drummond, 2017). A curcumina mostrou ser eficiente na redução do estresse oxidativo e atenuou a ansiedade em roedores (Da Silva Morrone et al., 2016). Além disso, estudos demostram que a curcumina apresenta efeito anti-inflamatório, principalmente devido à inibição da ciclo-oxigenase-2 (COX-2) e da produção de citocinas (Menon & Sudheer, 2007). Também reduziu a expressão gênica de citocinas pró-inflamatórias como o fator de necrose tumoral α (TNF-α), interleucina 1-β (IL-1β) e proteínas envolvidas na apoptose como fator nuclear kappa-B (NF-κB) e caspase-3 em modelo de depressão induzida por reserpina (Arora, Kuhad, Tiwari, & Chopra, 2011). A curcumina também foi capaz de reduzir níveis de inflamação, aumentar a neurogênese e normalizar níveis de estresse oxidativo em ratos expostos diariamente à estresse por restrição e a baixas doses de substâncias químicas como brometo de piridostigmina e N-N-dietil-mtoluamida (DEET), em um modelo de estresse pós-traumático (Kodali et al., 2018).

Apesar de seus efeitos benéficos, estudos indicam que a curcumina apresenta baixa biodisponibilidade (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). A biodisponibilidade de um composto pode ser melhorada através da micronização da matéria-prima através da aplicação da técnica de micronização por dióxido de carbono supercrítico (Aguiar et al., 2017). A micronização é uma técnica química verde adequada para preparar e modificar as propriedades de determinado composto. Essa ferramenta permite obter um tamanho de partícula grandemente reduzido, comparado com o material de partida, aumentando assim a sua solubilidade e a área de contato superficial (Bertoncello, Aguiar, Oliveira, & Siebel, 2018). Em um modelo de epilepsia induzida por pentilenotetrazol (PTZ) em peixe-zebra, a micronização da curcumina foi capaz de potencializar o efeito antiepiléptico quando comparada a uma curcumina não micronizada (convencional). Outro estudo mostrou que a micronização do resveratrol aumentou em 3,6 vezes os níveis do composto no plasma em pacientes com câncer colorretal quando comparado ao resveratrol não micronizado (Howells et al., 2011).

A curcumina é um composto natural com atividade antioxidante e aplicação promissora em diversos transtornos relacionados à oxidação celular, entretanto sua aplicação tem sido dificultada devido à baixa biodisponibilidade. Nesse contexto, o

objetivo desse trabalho foi comparar a atividade antioxidante da curcumina (CUR) e curcumina micronizada (CM) *in vitro.*

2. MATERIAL E MÉTODOS

2.1 CURCUMINA

A curcumina foi obtida da Sigma-Aldrich® (CAS: 458-37-7) e a micronização foi realizada no Laboratório de Termodinâmica e Tecnologia Supercrítica (LATESC) do Departamento de Engenharia Química e de Alimentos (EQA) da UFSC, através de parceria de pesquisa já estabelecida (Bertoncello et al., 2018). As diferentes preparações de curcumina foram diluídas em DMSO 1%. O ácido ascórbico, foi usado como controle positivo, obtido da Dinâmica® e diluído em água destilada. Dessa forma, os seguintes grupos experimentais foram testados: DMSO 1%; ácido ascórbico (0,0625, 0,25 e 1 g/L); curcumina (CUR 0,0625, 0,25 e 1 g/L) curcumina micronizada (CM 0,0625, 0,25 e 1 g/L). Essas concentrações foram baseadas em estudos anteriores (Bertoncello et al., 2018; Gilhotra & Dhingra, 2010; Xu et al., 2005). Todas as análises foram realizadas em duplicata com um n = 5.

2.2 ANÁLISES DA ATIVIDADE ANTIOXIDANTE

2.2.1 Ensaio de eliminação do radical 1,1-difenil-2-2-piciril-hidrazilo (DPPH)

A capacidade sequestradora de radicais livres foi avaliada pelo ensaio de DPPH baseado em Brand-Williams, Cuvelier, & Berset (1995) com modificações. 7,5 µL de amostra nas diferentes concentrações foram incubadas 24 h no escuro em temperatura ambiente com 7,5 µL de metanol e 285 µL de DPPH diluído (0,24 mg/mL de DPPH foi diluído em metanol até obter uma absorbância de 1,12 nm lida em λ 517 nm). DPPH e metanol, sem amostra foram utilizados como controle. Metanol foi utilizado como branco. Após incubação a absorbância foi lida em λ 517 nm. O cálculo para avaliar % inibição do radical DPPH foi realizado segundo a equação:

$$\% \text{ inibição do radical DPPH} = 100 \times \left[\frac{A_{controle} - (A_{amostra} - A_{branco})}{A_{controle}}\right]$$

2.2.2 Determinação do poder antioxidante redutor de ferro (FRAP)

O ensaio de FRAP foi realizado conforme Benzie & Strain (1996) com modificações. O método baseia-se na redução de Fe^{3+}(férrico) para Fe^{2+}(ferroso) por moléculas antioxidantes formando o complexo Fe^{2+}+ TPTZ de coloração azul, lido em λ 593 nm. 10 µL das diferentes concentrações das amostras foram incubadas a 37 °C durante 15 min com 300 µL da solução de trabalho contendo: 2,4,6-Tripiridil-Triazina (TPTZ) 10 mM + HCl 40 mM, $FeCl_3.6H_2O$ 20 mM e tampão acetato 300 mM (proporção 10:1:1). Brancos para cada amostra foram incubados sem TPTZ (substituído por água no reagente de trabalho), além do branco sem amostra. Dessa forma, a absorbância obtida na ausência de TPTZ foi subtraída daquela obtida com a presença do TPTZ. O aumento na absorbância devido à formação do complexo Fe^{2+} + TPTZ foi analisado.

2.2.3 Proteção contra a oxidação da glutationa (GSH)

A capacidade de prevenção da oxidação de GSH induzida pelo peróxido de hidrogênio (H_2O_2) foi medida pela presença de grupos sulfidrilas remanescentes de GSH que reagem com 5, 5'-ditiobis (ácido 2-nitrobenzóico) (DTNB), conforme Ellman (1959) com modificações. 10 µL de cada uma das amostras foram incubadas por 30 min. no escuro, a temperatura ambiente com meio de reação contendo 50 µL tampão de fosfato de potássio (200 mM, pH 6,4) e 42 µL de H_2O_2 (5 mM). Após o período de incubação, foram adicionados 12 µL do meio incubado à 238µL de DTNB, lido em 412 nm após 5 min. Amostras sem GSH foram utilizadas como branco da amostra e o meio de incubação sem amostra foram utilizados como controle. O cálculo para avaliar a porcentagem de grupos sulfidrilas remanescentes de GSH foi realizado segundo a equação:

$$\% \text{ grupos sulfidrilas remanescentes de GSH} = 100 \times \left[\frac{A_{controle} - (A_{amostra} - A_{branco})}{A_{controle}}\right]$$

2.2.4 Ensaio de desoxirribose

A capacidade de inibir a formação de radical hidroxila a partir do H_2O_2 via reação de fenton, foi avaliada pelo método de desoxirribose baseado em Halliwell, Gutteridge, & Aruoma (1987) com modificações. Ao qual a produção de $OH^{\cdot}$ leva a oxidação de desoxirribose e produção de malondialdeído (MDA), medido através das

substâncias reativas a ácido tiobarbitúrico (TBARS). Foram incubados 40 µL de KH_2PO_4 – KOH (50 mM, pH 7,4), 10 µL de desoxirribose (60 mM), 10 µL de $FeCl_3$ (1 mM), 10 µL de EDTA (1,04 mM), 10 µL de ácido ascórbico (2 mM), 10 µL de H_2O_2 (10 mM) e 10 µL das amostras nas diferentes concentrações. As misturas reacionais foram incubadas a 37 °C durante 1 h. Após, foram adicionados ao meio de incubação 100 µL de TBA a 1% e 100 µL de HCl a 25% e aquecido em banho-maria a 100 °C durante 15 min. A absorbância foi lida em λ 532 nm. Amostras sem desoxirribose foram utilizadas como branco da amostra e o meio de incubação sem amostra foram utilizados como controle. A porcentagem de inibição da formação de radical hidroxila foi realizado segundo a equação:

$$\% \text{ de inibição do radical hidroxila} = 100 \times \left[\frac{A_{controle} - (A_{amostra} - A_{branco})}{A_{controle}}\right]$$

2.3 ANÁLISE ESTATÍSTICA

A normalidade dos dados e homogeneidade das variâncias foram analisadas usando os testes de D'Agostino-Persone Levene, respectivamente. Os dados foram analisados por análise de variância de uma via (ANOVA) seguida pelo teste de Tukey e são apresentados como média ± erro padrão da média (E.P.M.). As diferenças foram consideras significativas quando $p<0,05$.

3. RESULTADOS

A figura 1 representa os resultados obtidos do ensaio de DPPH, mostrando que o poder antioxidante de inibição do radical DPPH da CM foi significativamente maior que a CUR na concentração de 1 g/L e equivalente ao controle positivo ácido ascórbico em todas as concentrações testadas ($p<0,0001$, $F(9,40)=228,2$). Porém, CUR foi mais efetiva que CM e ácido ascórbico na concentração de 0,0625 g/L e menos efetiva que o ácido ascórbico na concentração de 1 g/L. CM, CUR e ácido ascórbico em todas as concentrações testadas foram mais efetivas que o DMSO 1%.

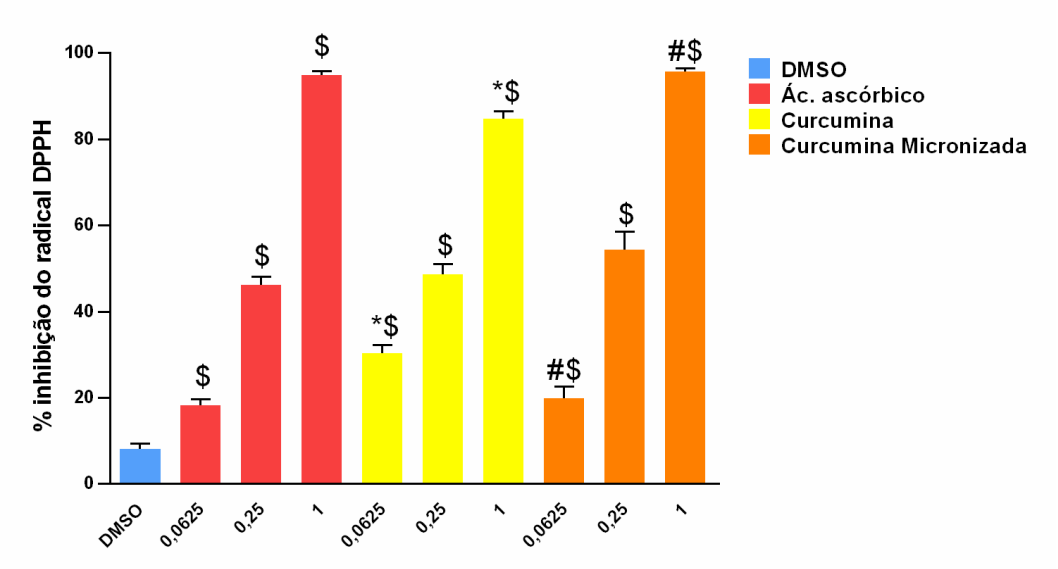

Figura 1. Efeito de diferentes tratamentos (DMSO, ácido ascórbico, curcumina e curcumina micronizada) sobre o percentual de inibição do radical DPPH *in vitro*. Os resultados são expressos como média ± E.P.M.*p<0,05 x ácido ascórbico (na mesma concentração). $^{\$}$p<0,05 x DMSO 1%. $^{\#}$p<0,05 x curcumina (na mesma concentração). ANOVA/Tukey (n=5). Concentrações mostradas em g/L.

A figura 2 mostra os resultados do ensaio de FRAP. O poder redutor do ferro no estado férrico para ferroso da CM foi significativamente maior que o da CUR nas concentrações de 0,25 e 1 g/L (p<0,0001, F(9,40)=316,2). Porém, o potencial redutor do ácido ascórbico foi maior que CM e CUR em todas as concentrações testadas. Tanto a CM quanto o ácido ascórbico foram melhores que o DMSO 1% em todas as concentrações testadas. Entretanto, CUR teve maior efeito redutor que DMSO 1% apenas nas concentrações de 0,25 e 1 g/L.

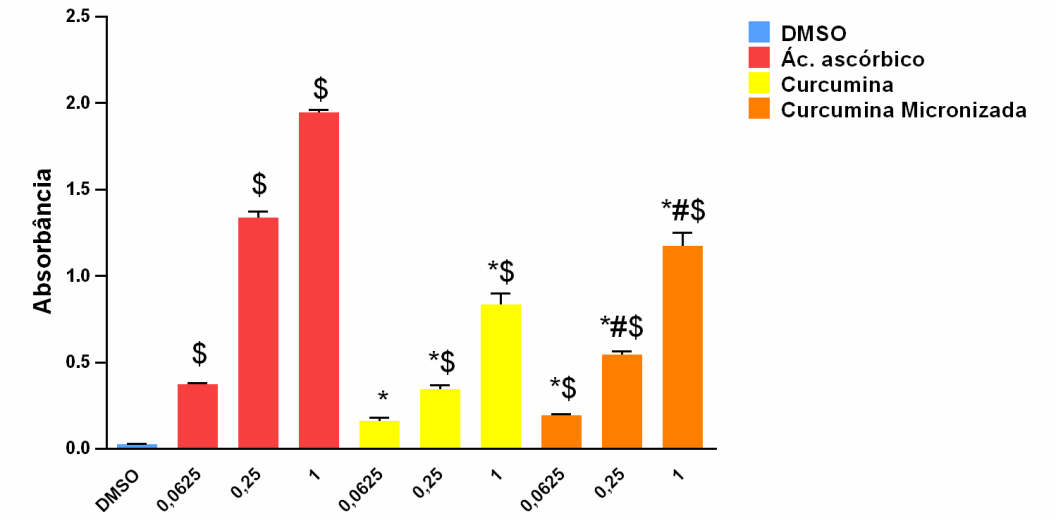

Figura 2. Efeito de diferentes tratamentos (DMSO, ácido ascórbico, curcumina e curcumina micronizada) sobre o potencial redutor de ferro (FRAP) *in vitro*. Os resultados são expressos como média ± E.P.M. *p<0,05 x ácido ascórbico (na mesma concentração). $^{\$}$p<0,05 x DMSO

1%. #p<0,05 x curcumina (na mesma concentração). ANOVA/Tukey (n=5). Concentrações mostradas em g/L.

A figura 3 representa a porcentagem de grupos sulfidrilas remanescentes de GSH, após indução de oxidação por peróxido de hidrogênio. CM, CUR, ácido ascórbico e DMSO foram capazes de prevenir a oxidação dos grupos sulfidrilas de GSH, visto que a porcentagem de grupos remanescentes foi em torno de 80%. Porém não houve diferenças significativas entre os tratamentos nas concentrações testadas.

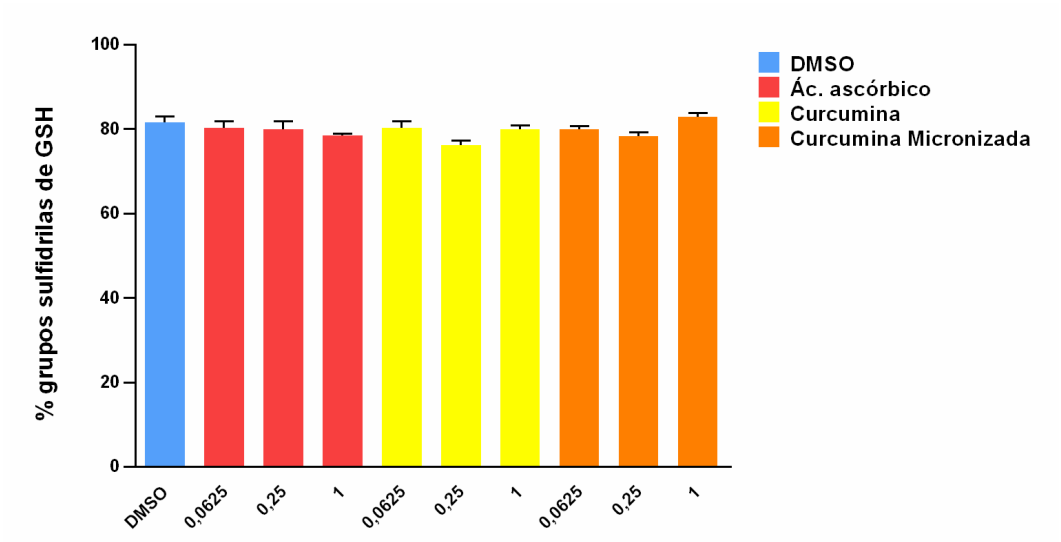

Figura 3. Efeito de diferentes tratamentos (DMSO, ácido ascórbico, curcumina e curcumina micronizada) sobre o percentual remanescente de sulfidrilas de GSH *in vitro*. Os resultados são expressos como média ± E.P.M. ANOVA/Tukey (n=5). Concentrações mostradas em g/L.

A figura 4 ilustra a porcentagem de inibição da formação do radical hidroxila. Tanto CM quanto CUR na concentração de 1 g/L foram mais efetivas que o ácido ascórbico em inibir a formação do radical hidroxila ($F(9,40) = 13,92$, $p<0,0001$). Porém o ácido ascórbico foi mais efetivo que CUR na concentração de 0,0625 g/L e CM nas concentrações de 0,0625 e 2,5 g/L. O DMSO 1% também foi mais efetivo em inibir a formação do radical hidroxila que a CM na concentração de 0,25 g/L.

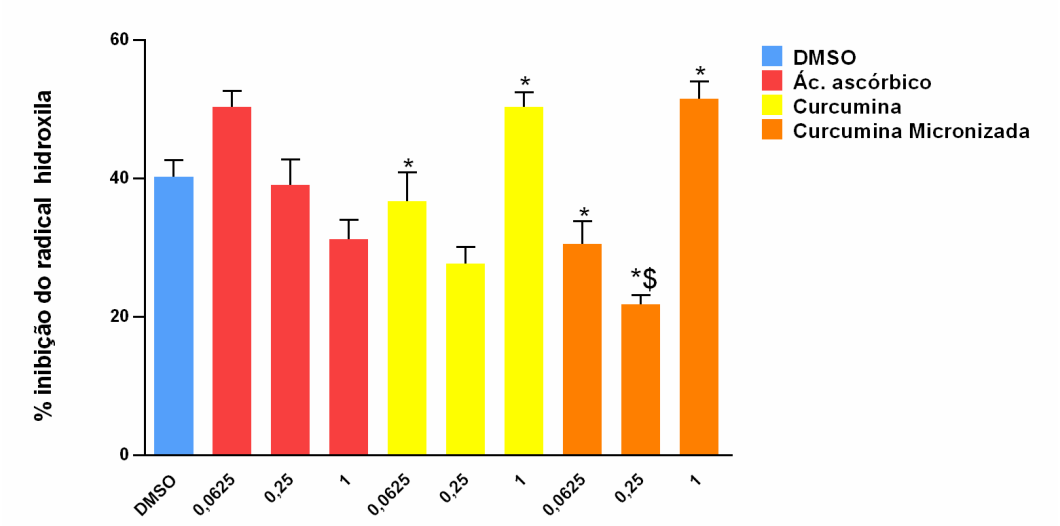

Figura 4. Efeito de diferentes tratamentos (DMSO, ácido ascórbico, curcumina e curcumina micronizada) sobre o percentual de inibição da formação do radical hidroxila *in vitro*. Os resultados são expressos como média ± E.P.M. *p<0,05 x ácido ascórbico (na mesma concentração). $^{\$}$p<0,05 x DMSO 1%. ANOVA/Tukey (n=5). Concentrações mostradas em g/L.

4. DISCUSSÃO

Nossos resultados mostram que a curcumina micronizada (CM) apresenta melhor efeito antioxidante do que a curcumina convencional (CUR). Isso pode ser verificado através dos testes de DPPH e FRAP que avaliam a capacidade doadora de elétrons, removedora de radicais livres e redutora respectivamente. Vários estudos demonstram que o efeito antioxidante da curcumina pode ser atribuído aos grupos fenólicos que possuem potencial antioxidante devido às suas propriedades como agentes redutores, doadores de hidrogênio, assim como inibidores de adsorção de oxigênio (Zheng et al., 2017). Resultados semelhantes já foram observados em diversos estudos, em que derivados da curcumina possuem melhores efeitos antioxidantes que a curcumina convencional (Landeros et al., 2017; Sahu, 2016; Singh et al., 2018). Entretanto, esse é o primeiro estudo que avalia o efeito antioxidante de uma preparação de curcumina micronizada.

O DPPH é um radical livre utilizado para avaliar a capacidade antioxidante de sua eliminação por determinado composto perante sua capacidade doadora de um átomo de hidrogênio ao DPPH, obtendo sua forma reduzida (Lee et al., 2009). No presente estudo observou-se que a CM apresentou atividade antioxidante de eliminação do radical DPPH equivalente à encontrada para o ácido ascórbico. Na concentração de 1 g/L, a CM apresentou capacidade antioxidante mais potente que a CUR. Enquanto o

ácido ascórbico teve maior capacidade de eliminação que a CUR nas concentrações de 1 g/L. Esses dados corroboram com encontrados na literatura que indicam que derivados da curcumina, como os componentes 4-(4-Hidroxifenil)-3,4-di-hidropirimidino-2 (1H)–ona curcumina e 4-(4-dimetilamino-fenil)-3,4-di-hidro-pirimidino-2 (1H)–tiona curcumina também parecem ser efetivos em eliminar radicais DPPH, apresentando taxas de eliminação maiores que 89% (Sahu, 2016).

O método FRAP é baseado na capacidade doadora de elétrons de um composto capaz de reduzir o ferro no estado férrico ao ferroso em solução, medido pelo acréscimo da absorbância (Halliwell, 1990). A capacidade doadora de elétrons da CM através da redução de ferro, mostrou-se mais eficaz nas concentrações de 0,25 e 1 g/L em comparação com a CUR. Um estudo que comparou a capacidade antioxidante de um novo derivado da curcumina (Cur–[G-2]–OH) demonstrou que esse composto é um terço mais eficaz na redução de ferro que a curcumina convencional, apresentando apenas um terço da fração líquida da curcumina em sua molécula, também possuindo atividade antioxidante mais potentes que o ácido ascórbico (Landeros et al., 2017). Os mecanismos responsáveis por esses resultados se devem pela capacidade doadora do átomo de hidrogênio bem como a transferência de elétrons (Li et al., 2015). Isso sustenta nossos resultados sugerindo que a capacidade de eliminação de radicais obtidos pelo ensaio de DPPH da CM relacionam-se às suas propriedades redutoras no ensaio de FRAP.

A proteção contra oxidação de GSH foi medida pelos grupos sulfidrilas remanescentes de GSH que reagem com DTNB após oxidação induzida por H_2O_2. O grupo sulfidrila presente no tiol da cisteína é o local ativo e responsável por suas funções protetoras contra o estresse oxidativo (Dasuri, Zhang, & Keller, 2013; Gandhi & Abramov, 2012). Sendo assim, sua oxidação leva a formação de dissulfetos de GSH e inativação da sua capacidade antioxidante, deixando o organismo mais susceptível a sofrer danos oxidativos. A CM e a CUR foram capazes de eliminar peróxidos em todas as concentrações testadas, com capacidade de inibição entorno de 80 %, sendo tão efetivas quanto o ácido ascórbico nesse ensaio. O que indica sua capacidade de transferir elétrons e assim prevenir a oxidação dos grupos sulfidrilas de GSH, fornecendo proteção contra o desequilíbrio no estado oxidativo do organismo.

No ensaio de desoxirribose, os radicais hidroxilas gerados pelo Fe^{2+}-ascorbato-EDTA-H_2O_2 (reação de Fenton) oxidam a desoxirribose havendo formação de MDA

(Halliwell, Gutteridge, & Aruoma, 1987). Dessa forma, é analisada a capacidade do composto em inibir a formação de radicais hidroxila ($OH^{\cdot}$) e, consequentemente, a inibição da formação de malondialdeído (MDA). No organismo, o radical hidroxila também reage com lipídeos de membrana e leva a formação de MDA. A inibição da peroxidação lipídica é considerada um índice importante de atividade antioxidante, pois é um ponto final de dano biológico que ocorre em várias doenças, incluindo transtornos neurodegenerativos (Uttara, Singh, Zamboni, & Mahajan, 2009). Nossos resultados indicam que tanto CM quanto CUR foram capazes de inibir a formação de radical hidroxila e CUR foi equivalente ao ácido ascórbico na concentração de 2,5 g/L. Indicando que a curcumina possui capacidade de eliminação de peróxidos, inibição da formação de radicais livres e assim pode prover proteção contra peroxidação lipídica, o que também tem sido justificado principalmente pela presença de grupos fenólicos na molécula. Estudos demonstram que a curcumina pode eliminar ânion superóxido e diminuir os níveis de MDA, e dessa forma, possui efeito neuroprotetor no hipocampo em ratos expostos a homocisteína (Ataie, Sabetkasaei, Haghparast, Moghaddam, & Kazeminejad, 2010).

5. CONCLUSÕES

A curcumina apresenta atividade antioxidante *in vitro* capaz de reduzir e remover radicais livres atuando como doadora ou na transferência de elétrons. A curcumina micronizada apresentou maior atividade antioxidante em relação à curcumina nas concentrações de 0,25 e 1 g/L, sendo mais eficiente em mecanismos envolvendo sua capacidade redutora e agindo como doadora de elétrons contra radicais livres e espécies reativas de oxigênio. Esse estudo mostra que a micronização é um processo capaz de alterar as características físico-químicas de compostos e, consequentemente, melhorar seu perfil de ação. Mais estudos são necessários para comparar as diferentes preparações de curcumina a fim de fornecer evidências para os estudos *in vivo*.

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5 CONCLUSÕES E PERSPECTIVAS

Demostramos que uma formulação micronizada de curcumina apresentou melhor perfil antioxidante *in vitro* em comparação a uma preparação convencional, sendo tão efetiva quanto o controle positivo ácido ascórbico. Assim, especulamos que a curcumina micronizada apresenta perfil físico-químico, solubilidade, biodisponibilidade e eficácia melhores do que a curcumina convencional. Porém, mais estudos *in vivo* são necessários para a elucidação completa desses efeitos. Pretende-se analisar os efeitos da CM sobre parâmetros comportamentais e bioquímicos em peixes-zebra a fim de determinar o potencial uso dessa preparação em transtornos mentais.

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ANEXO A – NORMAS DE PUBLICAÇÃO DA REVISTA JOURNAL OF FUNCTIONAL FOODS

JOURNAL OF FUNCTIONAL FOODS
AUTHOR INFORMATION PACK
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The *Journal of Functional Foods* aims to bring together the results of fundamental and applied research into healthy foods and biologically active food ingredients.
The Journal is centered in the specific area at the boundaries among food technology, nutrition and health welcoming papers having a good interdisciplinary approach.The journal will cover the fields of plant bioactives; dietary fibre, probiotics; functional lipids; bioactive peptides; vitamins, minerals and botanicals and other dietary supplements. Nutritional and technological aspects related to the development of functional foods and beverages are of core interest to the journal. Experimental works dealing with food digestion, bioavailability of food bioactives and on the mechanisms by which foods and their components are able to modulate physiological parameters connected with disease prevention are of particular interest as well as those dealing with personalized nutrition and nutritional needs in pathological subjects. Papers will cover topics such as new food bioactives; efficacy and safety of bioactive compounds, and other healthy food constituents using genomic, chemical and biochemical technologies.
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