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Jéssica Maurino dos Santos

Guazuma ulmifolia Lam. (Malvaceae) REDUZ ESTRESSE OXIDATIVO EM CÉLULAS SANGUÍNEAS E PREVINE CARDIOTOXIDADE INDUZIDA POR DOXORRUBICINA

Orientadora: Kely de Picoli Souza
Coorientadora: Ana Paula de Araújo Boleti
Grupo de pesquisa: Grupo de Estudos em Biotecnologia e Bioprospecção Aplicados ao Metabolismo – GEBBAM

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POR

JÉSSICA MAURINO DOS SANTOS

DISSERTAÇÃO APRESENTADA À UNIVERSIDADE FEDERAL DA GRANDE DOURADOS (UFGD), COMO PARTE DOS REQUISITOS EXIGIDOS PARA OBTENÇÃO DO TÍTULO DE MESTRE EM BIOLOGIA GERAL - ÁREA DE CONCENTRAÇÃO: "BIOPROSPECÇÃO".

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“O cientista não é o homem que fornece as verdadeiras respostas, é quem faz as verdadeiras perguntas”.

(Claude Lévi-Strauss)

Lista de abreviaturas

AAPH – 2,2`-azobis (2-amidinopropano) dicloridrato
APx – ascorbato peroxidase
CAT – catalase
DOX – doxorrubicina
DPPH – radical 2,2-difenil-1-picril-hidrazil
EcGU – extrato aquoso de casca de *Guazuma ulmifolia*
EfGU – extrato aquoso de folha de *Guazuma ulmifolia*
EROs – espécies reativas de oxigênio
GPx – glutationa peroxidase
GSH – glutationa
IC_{50} – capacidade inibitória de 50%
MDA – malondialdeído
MTT – brometo 3- (4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio
OMS – Organização Mundial da Saúde
PNPIC – Política Nacional de Práticas Integrativas e Complementares
PNPMF – Política Nacional de Plantas Medicinais e Fitoterápicos
PUFAs – ácidos graxos poli-insaturados
RENISUS – Relação de plantas medicinais de interesse ao Sistema Único de Saúde
SISBIO – Sistema de autorização e informação em biodiversidade
SOD – superóxido dismutase

RESUMO

O estresse oxidativo tem função chave em diversas doenças, bem como na cardiotoxicidade induzida por doxorrubicina (DOX) durante a quimioterapia, o que limita a aplicação clínica desta droga. Entretanto, a combinação de DOX e antioxidantes tem reduzido essa toxicidade. Neste estudo, avaliamos a capacidade antioxidante dos extratos aquosos da casca do caule (EcGU) e da folha (EfGU) de *Guazuma ulmifolia* em células sanguíneas submetidas a diferentes agentes oxidantes. Além disso, investigamos os efeitos do EcGU na toxicidade aguda e na prevenção da cardiotoxicidade induzida por DOX *in vivo*. Para isso, avaliamos a atividade antioxidante direta por captura do radical livre DPPH, inibição da peroxidação lipídica induzida por AAPH ou DOX em eritrócitos humanos e quantificação de EROs intracelular por sonda fluorescente DCFH-DA em células eritroleucêmicas K562 estimuladas com peróxido de hidrogênio. A viabilidade de células eritroleucêmicas K562 e leucócitos tratados com EcGU também foi avaliada pelo método de MTT na ausência ou presença de DOX. Além disso, foi avaliada a toxicidade aguda e o efeito cardioprotetor do EcGU em camundongos C57Bl/6 tratados com DOX.
Os EcGU e EfGU exibiram atividades antioxidantes por eliminação de radical livre, ação anti-hemolítica e diminuição do conteúdo de malondialdeído (MDA) gerado em eritrócitos humanos estimulados por agentes oxidantes. Ao apresentar globalmente melhor atividade antioxidante, o EcGU foi selecionado para os demais estudos. O EcGU apresentou redução de EROs intracelular em células K562 estimuladas por peróxido de hidrogênio, não interferiu na citotoxicidade da DOX em células K562 e não induziu morte celular de leucócitos, mas os protegeu contra a morte induzida por DOX, não apresentou toxicidade aguda *in vivo* e foi capaz de prevenir a geração de MDA no tecido cardíaco de camundongos tratados com DOX, demonstrando sua ação cardioprotetora. Em conjunto, os resultados mostram que o EcGU e EfGU são antioxidantes naturais e por isso, possíveis alternativas para o tratamento de doenças associadas ao estresse oxidativo e, em especial, o EcGU pode desempenhar papel adjuvante na quimioterapia com DOX.

Palavras-chave: antraciclina, malondialdeído, antioxidante, mutamba.

ABSTRACT

Oxidative stress has a key role in several diseases, as well as doxorubicin-induced cardiotoxicity (DOX) during chemotherapy, which limits the clinical application of this drug. However, the combination of DOX and antioxidants has reduced this toxicity. In this study, we evaluated the antioxidant capacity of *Guazuma ulmifolia* stem bark (GUEsb) and *Guazuma ulmifolia* leaves (GUEl) extracts in blood cells submitted to different oxidizing agents. In addition, we investigated the effects of GUEsb on acute toxicity and on the prevention of DOX-induced cardiotoxicity in vivo. For this, we evaluated the direct antioxidant activity by capturing the DPPH free radical, inhibiting lipid peroxidation induced by AAPH or DOX in human erythrocytes and quantifying intracellular EROs by fluorescence probe DCFH-DA in K562 erythroleukemic cells stimulated with hydrogen peroxide. The viability of K562 erythroleukemic cells and leukocytes treated with GUEsb was also evaluated by the MTT method in the absence or presence of DOX. In addition, the acute toxicity and cardioprotective effect of GUEsb in C57B1/6 mice treated with DOX were evaluated. The GUEsb and GUEl showed antioxidant activities by eliminating free radicals, anti-hemolytic action and reducing of the malondialdehyde (MDA) content generated in human erythrocytes stimulated by oxidizing agents. When presenting better overall antioxidant activity, GUEsb was selected for the other studies. GUEsb showed reduction of intracellular ROS in K562 cells stimulated by hydrogen peroxide, did not interfere in the cytotoxicity of DOX in K562 cells and did not induce leukocyte cell death but protected them against DOX-induced death, did not present acute toxicity in vivo and was able to prevent the generation of MDA in the cardiac tissue of mice treated with DOX, demonstrating its cardioprotective action. Together, the results show that GUEsb and GUEl are natural antioxidants and therefore possible alternatives for the treatment of diseases associated with oxidative stress and, in particular, GUEsb may play an adjuvant role in DOX chemotherapy.

Keywords: anthracycline, malondialdehyde, antioxidant, mutamba.

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SUMÁRIO

1 INTRODUÇÃO

O estresse oxidativo é uma condição desencadeada quando ocorre o desequilíbrio entre a quantidade de espécies reativas, como as de oxigênio (EROs), e a capacidade do organismo de neutralizar sua ação por meio do sistema de proteção antioxidante (MAURYA *et al*, 2016). Uma das consequências do estresse oxidativo é a peroxidação lipídica (AYALA; MUÑOZ; ARGÜELLES, 2014) que leva à perda de fluidez das membranas biológicas, inativação enzimática e danos aos ácidos nucleicos com consequente perda de suas funções biológicas (MORITA *et al*, 2016).

O estresse oxidativo está presente no quadro de diversas condições e doenças, dentre elas, diabetes (AOUACHERI *et al*, 2015), inflamação (BISWAS, 2016), doenças gastrointestinais (BHATTACHARYYA *et al*, 2014) e cardiovasculares (CSÁNYI & MILLER, 2014) bem como, no quadro de cardiotoxicidade induzida por uso de quimioterapia com antraciclinas (ARYAL & RAO, 2016).

A doxorrubicina (DOX) é um antibiótico da família das antraciclinas, altamente eficaz na quimioterapia contra cânceres sólidos e hematológicos (WEI *et al*, 2015), mas seu uso clínico é limitado por causar cardiotoxicidade (THANDAVARAYAN *et al*, 2015). Contudo, algumas combinações entre DOX e outros fármacos têm demonstrado serem capazes de reduzir esse efeito colateral do quimioterápico, como por exemplo, o dexrazoxane, que inibe a formação de complexos DOX-ferro e consequentemente reduz a formação de espécies reativas (LIPSHULTZ *et al*, 2004; LIPSHULTZ *et al*, 2012; LIPSHULTZ *et al*, 2015).

A partir desta constatação, estudos têm demostrado que a combinação de DOX e plantas medicinais com potencial antioxidante reduz a cardiotoxicidade induzida pelo quimioterápico, como por exemplo, *Camellia sinensis* (KHAN *et al*, 2014), *Capparis spinosa* (MOUSAVI *et al*, 2016) e *Rheim turkestanikum* (HOSSEINI & RAJABIAN, 2016). Cada vez mais, a bioprospecção de plantas que não causem toxicidade e desempenhem efeitos antioxidantes e potencial para evitar a peroxidação lipídica tem sido alvo de pesquisas (CASAGRANDE *et al*, 2014; CAMPOS *et al*, 2016; SANTOS *et al*, 2016).

Nesse sentido, é crescente o interesse por investigar outras plantas medicinais com potencial antioxidante. A *Guazuma ulmifolia* Lam. (Malvaceae), popularmente conhecida

como mutamba (LOPES *et al*, 2012) ou guácimo (VILLALOBOS *et al*, 2011), é utilizada na medicina tradicional na forma de infusão e decocto para o tratamento de inflamação (GÓMEZ-ESTRADA *et al*, 2011), doenças gastrointestinais (HEINRICH *et al*, 1992), hemorragias, estimulante para as contrações uterinas (MALDINI *et al*, 2013) e diabetes (BERENGUER *et al*, 2007).

Estudos farmacológicos têm indicado *in vitro* o potencial antidiabético (ALONSO-CASTRO & SALAZAR-OLIVO, 2008), e *in vivo*, os potenciais hipotensor e vaso-relaxante (MAGOS *et al*, 2008) da casca e antidiabético (ADNYANA *et al*, 2013), anti-hipercolesterolêmico (SUKANDAR *et al*, 2012) e gastroprotetor (BERENGUER *et al*, 2007) da folha dessa planta.

Adicionalmente, estudos fitoquímicos da folha, fruto (PATIL & BIRADAR, 2013) e casca do caule de *G. ulmifolia* (FELTRIN *et al*, 2012) identificaram compostos fenólicos que são descritos na literatura por sua atividade antioxidante (KOOLEN *et al*, 2013; AHMED *et al*, 2015; OROIAN & ESCRICHE, 2015). Estes compostos provavelmente contribuem para a atividade antioxidante identificada do óleo essencial (BOLIGON *et al*, 2013) e dos extratos etanólicos de casca (FELTRIN *et al*, 2012) e folha (MORAIS *et al*, 2016) de *G. ulmifolia*.

Buscando ampliar o conhecimento sobre os efeitos biológicos dos extratos aquosos da casca do caule e da folha de *G. ulmifolia*, avaliamos o potencial antioxidante em células sanguíneas submetidas a diferentes agentes oxidantes. E, investigamos os efeitos do extrato da casca do caule de *G. ulmifolia* na toxicidade aguda e na prevenção da cardiotoxicidade induzida por DOX *in vivo*.

2 REVISÃO DE LITERATURA

2.1 ESPÉCIES REATIVAS DE OXIGÊNIO E ESTRESSE OXIDATIVO

O oxigênio, além de ser essencial para a sobrevivência de organismos aeróbios, atua como aceptor final de elétrons na cadeia respiratória mitocondrial para a produção de energia na forma de adenosina trifosfato (BHATTACHARYYA *et al*, 2014). A cadeia respiratória mitocondrial é constituída de complexos enzimáticos (I – NADH–ubiquinona oxidorredutase, II– succinato desidrogenase, III – ubiquinol-citocromo *C* oxidorredutase e IV – citocromo *C* oxidase) que atuam em conjunto com a coenzima Q e a proteína citocromo *C* para reduzir o O_2 tetravalentemente para a formação de H_2O (BHATTACHARYYA *et al*, 2014). Nesse processo, são formadas espécies reativas como as de oxigênio (EROs), que também podem ser geradas na presença de fatores extrínsecos como fumaça de cigarro, ionizantes e radiações ultravioleta, pesticidas e ozônio (PISOSCHI & POP, 2015). Essas EROs são classificadas em radicalares, como o ânion superóxido ($O_2^{\bullet-}$) e radical hidroxila ($OH^{\bullet}$) e não radicalares, como o peróxido de hidrogênio (H_2O_2) (LUSHCHAK, 2014), por exemplo.

No ciclo redox, a primeira ERO formada a partir da redução do O2 é o O2•- (BIRBEN et al, 2012). Em seguida, o O2•- é dismutado a H2O2 que é convertido a OH• e posteriormente a água (H2O) pelas reações de Fenton ou diretamente convertido a H2O por ação do sistema antioxidante enzimático (Figura 1) (MAURYA et al, 2016).

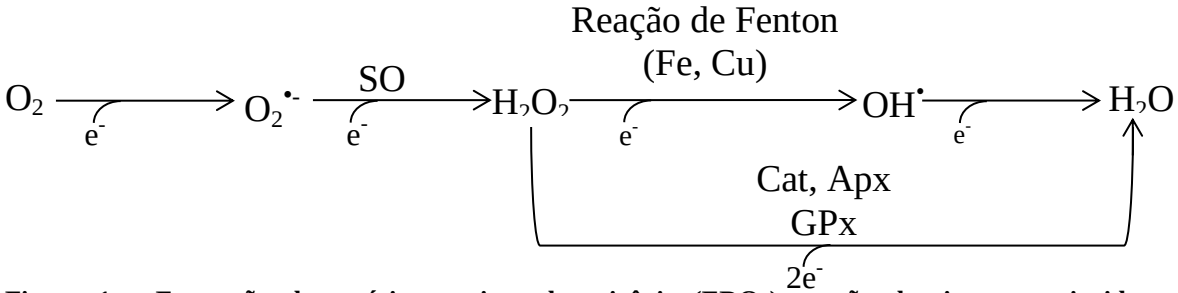

Figura 1 – Formação de espécies reativas de oxigênio (EROs) e ação do sistema antioxidante enzimático. No processo de respiração celular, o oxigênio molecular (O_2) é reduzido a ânion superóxido ($O_2^{\bullet-}$). Prontamente, o $O_2^{\bullet-}$ é reduzido a peróxidos de hidrogênio (H_2O_2) por meio da atuação da superóxido dismutase (SOD). O H_2O_2 por sua vez, pode ser reduzido a hidroxila ($OH^{\bullet}$) e posteriormente a água (H_2O) por meio da reação de Fenton ou ainda, reduzido diretamente a H_2O por meio da ação de enzimas antioxidantes endógenas, catalase (CAT), ascorbato peroxidase (APx) e glutationa peroxidase (GPx). Adaptado de MAURYA *et al*, 2016.

Em quantidades adequadas, as EROs atuam como sinalizadores, por exemplo, do sistema imunológico, e estão envolvidas tanto na regulação de vias metabólicas do organismo (LUSHCHAK, 2014), como na regulação da proliferação celular, expressão gênica e apoptose (PISOSCHI & POP, 2015). No entanto, em excesso, as EROs são capazes de reagir com biomoléculas, e causar danos à saúde do organismo (AYALA; MUÑOZ; ARGÜELLES, 2014; WANG; WANG; GU, 2016). Este desequilíbrio entre a produção de EROs e a capacidade ineficiente do organismo de neutralizar sua ação através do sistema de proteção antioxidante culmina no estresse oxidativo (MAURYA *et al*, 2016).

O estresse oxidativo, por sua vez, determina modificações na estrutura e função de proteínas, ácidos nucleicos e lipídios (PISOSCHI & POP, 2015), com consequente perda de suas funções biológicas e/ou desequilíbrio homeostático (PLAMPLONA & CONSTANTINI, 2011).

As EROs podem causar fragmentação da cadeia peptídica, alterar a carga elétrica, promover a ligação cruzada de proteínas e a oxidação de aminoácidos específicos e, assim, levar a um aumento da susceptibilidade à proteólise, causando mudanças conformacionais, desdobramento e degradação das proteínas, com consequente perda da função biológica (BIRBEN *et al*, 2012). Dentre os mecanismos de oxidação de proteínas, o mais comum é a catálise por metais. Isto requer a presença de íons metálicos como ferro ou cobre para se ligarem a sítios de proteínas, que em seguida reagem com H_2O_2 na reação de Fenton para gerar $OH^{\bullet}$, que por sua vez, oxida as cadeias laterais dos aminoácidos ou cliva o esqueleto da proteína (MØLLER; ROGOWSKA-WRZESINSKA; RAO, 2011).

No caso de enzima, quando oxidada, pode tornar-se inativa. Para enzimas que possuem metais no interior de seus sítios ativos, a oxidação é ainda mais provável (BIRBEN *et al*, 2012). Adicionalmente, diante de determinadas condições oxidantes, a atividade de algumas enzimas pode ser reduzida. A redução nos níveis de enzimas antioxidantes endógenas SOD e CAT, por exemplo, pode favorecer os danos oxidativos (MOMIN *et al*, 2012).

Os danos causados ao DNA são considerados os mais graves. As EROs são capazes de oxidar proteínas, que subsequentemente interagem com cadeias laterais de aminoácidos, causando danos aos ácidos nucleicos, na medida em que pode provocar reticulação DNA-proteína, quebra de cadeia e alteração na purina e estrutura de bases de pirimidina, tendo

como resultados mutações no DNA, o que, por sua vez, pode originar a oncogênese (PISOSCHI & POP, 2015).

Os lipídios também podem ser oxidados por EROs, sendo os ácidos graxos poli-insaturados (PUFAs) constituintes da membrana celular, ácido araquidônico (ω-6) e ácido docosa-hexaenóico (ω-3), os mais susceptíveis à oxidação (PISOSCHI & POP, 2015). O processo de oxidação dos lipídios é constituído por três etapas: iniciação, propagação e terminação (AYALA; MUÑOZ; ARGÜELLES, 2014). Na etapa de iniciação, as EROs como $OH^{\bullet}$ retiram o H^{+} dos PUFAs formando o radical lipídico (L·). Na fase de propagação, o L· reage rapidamente com o O_2 e forma um radical peroxila lipídico (LOO·) que abstrai um H^{+} a partir de uma outra molécula de lipídio, gerando hidroperóxidos lipídicos (LOOH) e um novo L· (que continua a reação em cadeia). Na terminação, os antioxidantes como a vitamina E doam um H^{+} à LOO· e formam um correspondente à vitamina E que reage com outro radical LOO formando produtos não radicalares. Uma vez que a peroxidação lipídica é iniciada, a propagação das reações em cadeia ocorrerá até produtos de terminação serem produzidos (AYALA; MUÑOZ; ARGÜELLES, 2014).

Durante esse processo, a oxidação de ω-3 e ω-6 forma o malondialdeído (MDA) (LARSSON *et al*, 2016), que é um reconhecido marcador de peroxidação lipídica (PISOSCHI & POP, 2015). O MDA parece ser o mais mutagênico dentre os produtos secundários formados durante a lipoperoxidação. Uma vez formado, o MDA pode ser enzimaticamente metabolizado, ou pode reagir com proteínas celulares e teciduais e com o DNA para formar complexos que resultam em danos biomoleculares (AYALA; MUÑOZ; ARGÜELLES, 2014).

Como consequência da peroxidação lipídica, ocorre redução da fluidez da membrana, facilitando a troca de fosfolipídios entre a bicamada lipídica, aumentando o fluxo de substâncias que normalmente não atravessariam, a não ser por canais específicos (como por exemplo, cálcio e potássio), além de inativar receptores, enzimas e canais iônicos (HALLIWELL, 2006; GILL & TUTETA, 2010), o que tem sido relacionado com a patogênese de várias doenças (MORITA *et al*, 2016).

Assim, já é relatado que o estresse oxidativo está presente no envelhecimento (MAURYA *et al*, 2016) e em diversas doenças como, diabetes *mellitus* (AOUACHERI *et al*, 2015), doenças inflamatórias (BISWAS, 2016), renais (MARTINÉZ-CECILIA *et al*, 2016), cardiovasculares (MYOREN *et al*, 2016), neurodegenerativas (PISOSCHI & POP, 2015; PINGITORE *et al*, 2015) e câncer (KANG *et al*, 2016).

Além disso, existem fármacos como as antraciclinas administradas na quimioterapia, que diretamente podem promover a ruptura da sinalização redox prejudicando a eliminação de EROs, ou indiretamente, reduzindo os níveis intracelulares de antioxidantes e, assim, em ambos os casos gerar estresse oxidativo e morte de células cancerígenas por apoptose (MUT-SALUD *et al*, 2016). No entanto, o estresse oxidativo causado por quimioterápicos pode também promover toxicidade em tecidos não alvo (SEEBACHER *et al*, 2016), pois, via de regra, essas drogas são administradas via oral ou intravenosa, espalhando-se rapidamente para outros órgãos por meio da corrente sanguínea (KANEHIRA *et al*, 2016). Alguns efeitos adversos são característicos em pacientes em processo de quimioterapia, como diarreia, leucopenia, nefrotoxicidade e cardiotoxicidade (KANEHIRA *et al*, 2016).

A doxorrubicina (DOX) é um medicamento da família das antraciclinas, extraído e posteriormente sintetizado a partir da bactéria gram positiva *Streptomyces peucetius var. caesius* e rotineiramente utilizado no tratamento de vários cânceres (MALLA *et al*, 2010; THORN *et al*, 2011), incluindo o de mama (CHAKRAVARTY *et al*, 2016), pulmão (KANEHIRA *et al*, 2016), estômago (MORIKAWA *et al*, 2015), tiroide (SHERMAN *et al*, 2011), linfoma não-Hodgkin (CUNNINGHAM *et al*, 2013), mieloma múltiplo (CRUSOE *et al*, 2014), leucemia aguda (ORTIZ-LAZARENO *et al*, 2014), entre outros.

Existem dois mecanismos propostos pelos quais a DOX atua na célula cancerígena (1) intercalação e ruptura na reparação de DNA mediada por inibição da topoisomerase-II e (2) geração de EROs e o seus danos às membranas celulares, proteínas e DNA (THORN *et al*, 2011), o que culmina em morte celular por apoptose (MUT-SALUD *et al*, 2016). Resumidamente, na presença de NADH-desidrogenase a DOX é reduzida a semiquinonas no complexo mitocondrial respiratório I. Quando em condições aeróbias, estas são instáveis e facilmente reduzem o O_2 para $O2^{\bullet-}$. Posteriormente, o ciclo redox produz H_2O_2 e $OH^{\bullet}$. Reações entre Fe e DOX também podem gerar moléculas reativas ao catalisar a reação de Fenton (VOLKOVA & RUSSELL, 2011). O excesso de EROs, por sua vez, pode culminar no estresse oxidativo, causar a lipoperoxidação das membranas, danos ao DNA, e por fim desencadear vias apoptóticas de morte celular (THORN *et al*, 2011; MORIKAWA *et al*, 2015). Além disso, a DOX pode induzir cardiotoxicidade alterando o metabolismo de lipídeos (ABUSHOUK *et al*, 2017). Pacientes com câncer tratados com DOX apresentaram maiores níveis de lipoproteína de baixa densidade (LDL) e redução de

lipoproteínas de alta densidade (HDL) (SHARMA *et al*, 2016). Abdel-aleem *et al*. (1997) sugeriram que a cardiotoxicidade induzida por DOX em miócitos pode ser parcialmente explicada pela inibição da oxidação de ácidos graxos cardíacos, causando desequilíbrio energético e insuficiência cardíaca congestiva.

Em conjunto, o aumento de estresse oxidativo, a peroxidação lipídica, a redução dos níveis de antioxidantes (CHATTERJEE *et al*, 2010) bem como a ativação de vias apoptóticas ocasionam a perda de miócitos funcionais e lesão irreversível do tecido cardíaco, uma vez que essas células não se regeneram (ZHAO & ZANG, 2017). Assim, a aplicação clínica de DOX torna-se restrita (MOUSAVI *et al*, 2016). Desse modo, compostos que atuem como antioxidantes, minimizando o estresse oxidativo e consequentemente a cardiotoxicidade causada pelo uso de DOX, são frequentes alvos de estudos (KANG *et al*, 2011; MOMIN *et al*, 2012; KHAN *et al*, 2014; DONG *et al*, 2014).

2.2 ANTIOXIDANTES

Caracteriza-se como antioxidante qualquer composto que, quando presente em uma menor concentração, comparada com a de um substrato oxidável, é capaz de atrasar ou evitar a oxidação causada por este agente (KASOTE *et al*, 2015). Os antioxidantes podem ser de duas origens: endógena, que compreende enzimas e componentes não enzimáticos, ou exógena, quando adquiridos através da dieta (PISOSCHI & POP, 2015).

2.2.1 Antioxidantes endógenos

Os antioxidantes endógenos enzimáticos são a primeira linha de defesa antioxidante dos organismos, na qual diferentes enzimas e produtos não enzimáticos desempenham papéis chave na manutenção do balanço redox (MAURYA *et al*, 2016).

A superóxido dismutase (SOD) é a principal enzima a atuar no sistema antioxidante endógeno (MAURYA *et al*, 2016). Três tipos dessa enzima são encontrados nos tecidos de mamíferos. A SOD1 utiliza como cofator cobre (Cu) e zinco (Zn) e encontra-se no citosol,

a SOD2 utiliza o manganês (Mn) e encontra-se na matriz mitocondrial e a SOD3 utiliza Cu e Zn e é extracelular. A SOD atua dismutando o $O_2^{\bullet-}$ e transformando-o em H_2O_2. A catalase (CAT) é expressa em elevadas concentrações no fígado e eritrócitos, e reduz o H_2O_2 a H_2O (PISOSCHI & POP, 2015). Já a glutationa peroxidase (GPx) utiliza o selênio como cofator (BRIGELIUS-FLOHÉ & MAIORINO, 2013) e catalisa a redução tanto de H_2O_2, quanto de $OH^{\bullet}$ a H_2O (PISOSCHI & POP, 2015) (Figura 1).

Entre os fatores antioxidantes não enzimáticos tem-se a glutationa (GSH) que é sintetizada a partir da combinação de glutamina, glicina e cisteína (MAURYA *et al*, 2016). Seus grupos tióis são oxidados a ligações dissulfeto, o que torna a GSH um excelente doador de elétrons (PISOSCHI & POP, 2015), e ainda, um substrato para a GPx neutralizar o H_2O_2 (MAURYA *et al*, 2016). Outro exemplo é a ubiquinona, que atua como transportador de elétrons e de íons de H^+ nas mitocôndrias (PETROVA *et al*, 2014) tornando-se essencial para respiração celular, além de inibir a peroxidação lipídica (PISOSCHI & POP, 2015).

2.2.2 Antioxidantes exógenos

Os antioxidantes exógenos podem ser ainda, sintéticos ou naturais. Os sintéticos são idênticos aos naturais, no entanto sintetizados industrialmente. São compostos puros, bem caracterizados e relativamente acessíveis financeiramente. Já os naturais são produzidos por microrganismos, fungos, animais e principalmente por plantas (RADOMSKA-LESNIEWSKA *et al*, 2016).

Os antioxidantes sintéticos como hidroxitolueno butilado (BHT), hidroxianisol butilado (BHA) e terc-butil hidroquinona (TBHQ) são utilizados nas indústrias alimentícia, cosmética e farmacêutica (YEHYE *et al*, 2015) para retardar ou prevenir a deterioração oxidativa. No entanto, estes antioxidantes estão relacionados a riscos para a saúde, pois possuem alta volatilidade e instabilidade na presença de elevadas temperaturas e relatos no desenvolvimento de doenças cardíacas e carcinogênese (LOBO *et al*, 2010; MARANGONI & MOURA, 2011). Por isso, o uso de antioxidantes sintéticos é restrito, definido e monitorado nacional (BRASIL, 2007) e internacionalmente (FDA, 2015). Neste

contexto, há uma tendência para o uso dos antioxidantes naturais em substituição aos sintéticos (ZHANG *et al*, 2010).

As vitaminas, minerais e alguns metabólitos secundários, como alguns compostos fenólicos, podem desempenhar atividades antioxidantes e consequentemente reduzir a incidência de danos oxidativos (SKROVANKOVA *et al*, 2015). Dentre as vitaminas com maior potencial antioxidante destacam-se o ácido ascórbico (AA) e o tocoferol. O AA é mencionado como o mais importante antioxidante hidrofílico, sendo eficaz na eliminação de $O2^{\bullet-}$, $OH^{\bullet}$, H_2O_2, tanto que sua deficiência é característica no processo de estresse oxidativo (VISWANATHA *et al*, 2011). No entanto, quando em concentrações elevadas (1000 mg Kg^{-1}) é altamente reativo e pode tornar-se oxidante (OROIAN & ESCRICHE, 2015). Já o tocoferol é um antioxidante lipossolúvel pertencente ao grupo de compostos químicos tocoferóis e tocotrienois que atua como doador de elétrons ou quelante (OROIAN & ESCRICHE, 2015), inibindo a peroxidação lipídica (MILLAO & UQUICHE, 2016).

Os minerais também desempenham papel fundamental na homeostase do organismo, uma vez que atuam como cofatores enzimáticos das enzimas antioxidantes (BRIGELIUS-FLOHÉ & MAIORINO, 2013; PISOSCHI & POP, 2015).

Os compostos fenólicos são produtos do metabolismo secundário de plantas derivados do ácido chiquímico e/ou mevalônico (KABERA *et al*, 2014) são sintetizados em condições de estresse para a planta. Desempenham papel vital no crescimento e propagação ao proteger os tecidos vegetais contra danos causados por patógenos e condições adversas à sobrevivência (OSZMIAŃSKI *et al*, 2015)**.** Os compostos fenólicos desempenham importante papel na prevenção e/ou tratamento de diversas doenças (OROIAN & ESCRICHE, 2015) por possuírem ação antimicrobiana (KOOLEN *et al*, 2013), hipotensiva e vasorelaxante (MAGOS *et al*, 2008), hipolipidêmica (MAMUN *et al*, 2013), citotóxica (CASAGRANDE *et al*, 2014), antioxidante (CAMPOS *et al*, 2016; SANTOS *et al*, 2016; ENCARNAÇÃO *et al*, 2016), entre outras.

A classe de compostos fenólicos inclui ácidos fenólicos simples (benzoico e derivados de ácido cinâmico), estilbenos, lignanas, ligninas e flavonoides (BLAINSKI; LOPES; MELLO, 2013). São capazes de eliminar EROs (AHMED *et al*, 2015) através da doação de H^{+} ou elétrons para formar moléculas intermediárias estáveis (KOOLEN *et al*, 2013). Os flavonoides são os mais comuns e amplamente distribuídos dentro da classe dos

compostos fenólicos. São pigmentos naturais de variável estrutura fenólica com anéis aromáticos A e B unidos por três carbonos que formam um anel heterocíclico, denominado anel C (KUMAR & PANDEY, 2013). São, ainda, divididos em subclasses, sendo: flavonas, flavanonas, flavonois, catequinas, antocianinas e isoflavonas as mais representativas (OROIAN & ESCRICHE, 2015). Em geral a atividade antioxidante dos flavonoides é dependente da classe estrutural, grau de hidroxilação e polimerização (KUMAR & PANDEY, 2013). São capazes de eliminar EROs e quelar ou se complexar com íons metálicos, características atribuídas a presença do anel aromático (KUMAR; MISHRA; PANDEY, 2013). Estes podem, ainda, atuar modulando a atividade do sistema antioxidante enzimático (KUMAR & PANDEY, 2013).

Em geral, compostos bioativos podem desempenhar atividades farmacológicas isoladamente ou, ainda, em sinergismo (TIWARI; ROY; TIWARI, 2015). Assim, a busca por caracterizar o potencial farmacológico bem como a constituição fitoquímica de plantas medicinais tornou-se alvo de muitos estudos (AHMED *et al*, 2015; CALIXTO-JÚNIOR, *et al*, 2016; SANTOS *et al*, 2016; CAMPOS *et al*, 2016).

2.3 PLANTAS MEDICINAIS

O uso de plantas medicinais como alternativa terapêutica tem sido praticado a milhares de anos. As civilizações antigas desenvolveram e aprimoraram seus próprios sistemas terapêuticos, fazendo uso dos recursos biológicos locais, com base em observações empíricas e suas inferências. Este conhecimento, no passado, representou a única opção terapêutica para muitas comunidades e, hoje, ainda é a única alternativa para várias comunidades isoladas (TRIBESS *et al*, 2015). Estima-se que cerca de 80% da população mundial é dependente de práticas da medicina tradicional para alguns aspectos em cuidados na saúde primária, o que levou a Organização Mundial da Saúde (OMS) a documentar o uso de plantas medicinais por nativos de diferentes partes do mundo (OMWENGA *et al*, 2015).

Devido ao papel na gestão de várias doenças crônicas, as drogas de origem vegetal, hoje, são a grande aposta dos pesquisadores na procura de fontes alternativas de medicamentos alopáticos (KIFAYATULLAH *et al*, 2015). Assim, as pesquisas científicas

buscam validar o uso popular de plantas medicinais (TRIBESS *et al*, 2015) utilizando informações obtidas em estudos etnobotânicos a respeito da identificação correta, utilização terapêutica e o uso racional de recursos oriundos da flora, para assim, contribuir com a descoberta de novos compostos e fornecer uma base para futuras pesquisas (RIBEIRO *et al*, 2014; TRIBESS, *et al*, 2015).

Recentemente, a combinação de DOX com extratos de diversas espécies incluindo, *Rheim turkestanikum* (HOSSEINI & RAJABIAN, 2016) e *Capparis spinosa* (MOUSAVI *et al*, 2016) foi capaz de reduzir a cardiotoxicidade induzida por DOX em modelo celular de cardiomioblastos murino (H9c2). Em modelo animal, a combinação de DOX com extratos advindos das plantas medicinais: *Vaccinium macrocarpon* (ELBERRY *et al*, 2010), *Ixora coccínea* (MOMIN *et al*, 2012), *Camellia sinensis* (KHAN *et al*, 2014) *e Curcuma longa* (EL-SAYED *et al*, 2011), foi capaz de atenuar a cardiotoxidade, melhorar a atividade de enzimas antioxidantes endógenas e melhorar parâmetros bioquímicos relacionados a cardiotoxicidade.

Por outro lado, a partir do termo “uso tradicional de plantas medicinais”, as pessoas estão sujeitas a entender que "natural" significa "seguro" e, consequentemente, acreditam que o consumo sem orientação não traz riscos à saúde. Entretanto, esse pensamento pode ser um equívoco, uma vez que as plantas possuem diversos constituintes, dentre os quais alguns são altamente tóxicos, e erros na identificação da espécie ou na quantidade de uso também podem levar à toxicidade (ENCARNAÇÃO *et al*, 2016). Nesse sentido, torna-se importante a realização de ensaios de toxicidade para garantir que a planta seja segura para consumo e desenvolvimento de novas drogas (PARASUMARAN, 2011).

O Brasil conta, desde 2006, com a Política Nacional de Práticas Integrativas e Complementares (PNPIC) e a Política Nacional de Plantas Medicinais e Fitoterápicos (PNPMF), que incentivam a investigação e desenvolvimento de medicamentos à base de plantas que priorizem tanto o acesso seguro e eficaz a estes medicamentos quanto à proteção da biodiversidade. Além disso, estes documentos promoveram à inclusão de plantas medicinais como tratamento no sistema público de saúde (AMENI *et al*, 2015). Com isso, a Relação de Plantas Medicinais de Interesse ao Sistema Único de Saúde (RENISUS) conta com 71 espécies (BRASIL, 2009).

A bioprospecção de plantas com potencial farmacológico é de grande interesse, pois cerca de 25% dos medicamentos prescritos em todo o mundo são de origem vegetal. (TRIBESS *et al*, 2015). Com isso, o interesse por medicamentos derivados de plantas

medicinais, ou os chamados fitoterápicos, aumentou de forma significativa em todo o mundo (DUTRA *et al*, 2016). Este interesse é visto especialmente em países desenvolvidos, como alguns países europeus e nos Estados Unidos, nos quais os fitoterápicos rendem milhões ao ano (DUTRA *et al*, 2016). Nesse aspecto, o Brasil possui uma grande vantagem, a maior biodiversidade do planeta (RIBEIRO *et al*, 2014).

O Brasil é o quinto maior país do mundo, tanto em termos geográficos quanto populacionais. Possui uma incrível riqueza de espécies de microrganismos, animais e plantas, distribuídos em seus vários ecossistemas de diferentes características climáticas (equatoriais, subtropicais e temperadas). Neste cenário diversificado encontra-se grande variedade de espécies (BARBIERI *et al*, 2014) que, juntamente com os diferentes grupos étnicos favorece um rico conhecimento sobre a vegetação (RIBEIRO *et al*, 2014).

Cerca de 20% do total de espécies do planeta estão distribuídas no território brasileiro (RIBEIRO *et al*, 2014) ao longo de seus seis biomas: Amazônia, Caatinga, Mata Atlântica, Pantanal, Pampa e Cerrado. O Cerrado (savana brasileira) é o segundo bioma mais extenso da América do Sul e tem sido nomeado como um dos *hotspots* mundiais de biodiversidade (BEUCHLE *et al*, 2015). Sua extensão ocupa cerca de 24% das terras brasileiras e possui uma das floras mais ricas do mundo, onde cerca de 35% das espécies vegetais são endêmicas (RIBEIRO *et al*, 2014). Dentre as plantas encontradas no Cerrado brasileiro destacam-se as florações da família Malvaceae.

2.3.1 Malvaceae

A família Malvaceae contém mais de 200 gêneros com cerca de 2300 espécies. É caracterizada por possuir plantas com flores. A principal utilização econômica das plantas dessa família é como fornecedora de fibras naturais, sendo importantíssimas para as indústrias têxteis. Também são usadas para alimentos, bebidas, madeira, horticultura e medicina tradicional (RIZK & SOLIMAN, 2014). As plantas desta família têm sido relatadas por possuírem atividades antioxidantes *in vitro* (OLIVEIRA *et al*, 2012), diurética (ALARCÓN-ALONSO *et al*, 2012), anti-inflamatória, analgésica, imunoestimuladora (ROSA *et al*, 2014), hipoglicemiante e hipolipidêmica em animais

experimentais (MAMUN *et al*, 2013) bem como hipolipidêmica em humanos (SABZGHABAEE *et al*, 2013).

Dentre as plantas pertencentes à Malvaceae, encontra-se a *Guazuma ulmifolia* Lam. (Figura 2) (CALIXTO-JÚNIOR *et al*, 2016), que primeiramente foi classificada como pertencente à Sterculiaceae, tanto que alguns estudos (VILLALOBOS *et al*, 2011; MUQARRABUN & AHMAT, 2015) ainda a mencionam como tal. A *G. ulmifolia* é uma árvore de porte médio (VILLALOBOS *et al*, 2011), cresce até 30 m de altura e pode atingir entre 30 e 40 cm de diâmetro. Possui copa arredondada. Suas folhas são cobertas por pequenos pelos em formato de estrela. As flores são pequenas e de coloração marrom-amarelada. Os frutos possuem cápsulas em formato elíptico com o interior repleto de pequenas sementes (RAMAKRISHNA *et al*, 2014).

A *G. ulmifolia* é popularmente conhecida como mutambo, mutamba, fruta-do-macaco, embira (SOBRINHO *et al*, 2012) ou guácimo (VILLALOBOS *et al*, 2011). É encontrada em alguns países da Ásia: Índia (KANERIA *et al*, 2009), Indonésia (ISWANTINI *et al*, 2011), e em vários países da América Latina como, México (GÁRCIA-ESTRADA; PEÑA-SÁNCHEZ; COLÍN-MARTÍNEZ, 2015), Colômbia (GÁRCIA-ATENCIA; MARTÍNEZ-HERNÁNDEZ; PARDO-LOCARNO, 2015), Costa Rica (VILLALOBOS *et al*, 2011), Bolívia (DEVISSCHER *et al*, 2016) e Brasil (CALIXTO-JUNIOR *et al*, 2016).

Em território brasileiro, essa espécie pode ser encontrada em vários estados como, Amazonas, Bahia, Distrito Federal (BRAGA *et al*, 2012), Goiás (SOBRINHO *et al*, 2012), Minas Gerais (LOPES *et al*, 2015), Pernambuco (KIMMEL *et al*, 2010), São Paulo, Tocantins (BRAGA *et al*, 2012), Paraná e Mato Grosso do Sul (SLUSARSKI & SOUZA, 2012).

Ela é usada como planta ornamental em vários países tropicais. Suas sementes produzem óleos aromáticos que são usados pela indústria cosmética (SCALON *et al*, 2011). A madeira é utilizada como combustível e carvão, e as folhas como forragem para o gado (RAMAKRISHNA *et al*, 2014). Na medicina tradicional, é utilizada para o tratamento de doenças respiratórias (SCALON *et al*, 2011). Sua casca é usada no tratamento de diarreia, hemorragias, febre, doenças inflamatórias e como estimulante para as contrações uterinas. O chá das folhas secas é utilizado em alguns países para doenças gastrointestinais, disenteria (MALDINI *et al*, 2013) e diabetes (BERENGUER *et al*, 2007).

Estudo fitoquímico caracterizou a presença de diferentes metabólitos secundários nas folhas e frutos de *G. ulmifolia*, dentre eles: alcaloides, saponinas, terpenos, flavonoides, taninos, glicosídeos para ambos e esteroides apenas nas folhas (PATIL & BIRADAR, 2013). Na casca do caule foram identificados compostos fenólicos como os flavonoides (FELTRIN *et al*, 2012) e os taninos condensados (MALDINI *et al*, 2013). O óleo essencial possui como compostos majoritários: timol, carvacrol e eugenol (BOLIGON *et al*, 2013).

Estudos *in vitro* observaram o efeito antidiabético do extrato aquoso da casca de *G. ulmifolia* em adipócitos (ALONSO-CASTRO & SALAZAR-OLIVO, 2008), o extrato etanólico das folhas foi capaz de inibir a lipase pancreática, (ISWANTINI *et al*, 2011) e o óleo essencial possui atividade antioxidante (BOLIGON *et al*, 2013). Em estudos *in vivo*, o extrato etanólico de folhas e flores apresentou um efeito gastroprotetor (BERENGUER *et al*, 2007). Magos *et al* (2008), ao administrar procianidinas isoladas a partir do extrato de acetona da casca *G. ulmifolia*, observaram um efeito hipotensor e vaso-relaxante em ratos.

Apesar das folhas, cascas e raízes serem empregadas na medicina tradicional em todas as regiões onde é encontrada, sua eficácia e segurança não foram, ainda, totalmente comprovadas cientificamente (SILVA; MARINHO; ANSELMO, 2012).

Figura 2 – (A) *Guazuma ulmifolia* Lam. (B) casca do caule. (C) folhas e (D) frutos.
(Fonte: Santos J. M, 2015).

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4 OBJETIVOS

4.1 OBJETIVO GERAL

Avaliar a capacidade antioxidante dos extratos aquosos da casca do caule e da folha de *G. ulmifolia* em células sanguíneas humanas submetidas a diferentes agentes oxidantes. E, investigar os efeitos do extrato de *G. ulmifolia* que apresentar melhor potencial antioxidante na toxicidade aguda e na prevenção da cardiotoxicidade induzida por DOX *in vivo*.

4.2 OBJETIVOS ESPECÍFICOS

- Quantificar os compostos fenólicos e flavonoides nos extratos aquosos de casca (EcGU) e folha (EfGU) de *G. ulmifolia*;
- Avaliar a capacidade antioxidante de EcGU e EfGU na captura direta de radical livre DPPH;
- Avaliar a atividade hemolítica de EcGU e EfGU;
- Avaliar a capacidade de EcGU e EfGU em proteger eritrócitos humanos contra o estresse oxidativo induzido por AAPH e doxorrubicina;
- Verificar qual dos extratos de *Guazuma ulmifolia* (EcGU ou EfGU) apresentou o melhor potencial antioxidante *in vitro* e com este:
- Avaliar a capacidade de inibir EROs intracelular;
- Avaliar a capacidade de interferir na atividade anticâncer de doxorrubicina em linhagem celular eritroleucêmica K562;
- Avaliar a toxicidade em leucócitos humanos;
- Avaliar a capacidade de proteger os leucócitos humanos contra a morte induzida por doxorrubicina;
- Avaliar a toxicidade aguda em camundongos fêmeas C57Bl/6;
- Avaliar a capacidade de proteger o tecido cardíaco em modelo de cardiotoxicidade induzida por doxorrubicina em camundongos C57Bl/6.

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Research Article

Guazuma ulmifolia Lam. Decreases Oxidative Stress in Blood Cells and Prevents Doxorubicin-Induced Cardiotoxicity

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Doxorubicin (DOX) is an efficient chemotherapeutic agent, but its clinical application is limited by its cardiotoxicity associated with increased oxidative stress. Thus, the combination of DOX and antioxidants has been encouraged. In this study, we evaluated (I) the chemical composition and antioxidant capacity of aqueous extracts from *Guazuma ulmifolia* stem bark (GUEsb) and leaves (GUEl) in 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, 2,2′-azobis(2-amidinopropane) dihydrochloride- (AAPH-) or DOX-induced lipid peroxidation inhibition in human blood cells, and intracellular reactive oxygen species (ROS) quantification using the fluorescent probe dichloro-dihydro-fluorescein diacetate (DCFH-DA) in K562 erythroleukemia cells incubated with GUEsb and stimulated with hydrogen peroxide; (II) the viability of K562 cells and human leukocytes treated with GUEsb in the absence or presence of DOX using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay; (III) the acute toxicity of GUEsb; and (IV) the cardioprotective effect of GUEsb in C57Bl/6 mice treated with DOX. The chemical composition indicated the presence of flavan-3-ol derivatives and condensed tannins in GUEsb and glycosylated flavonoids in GUEl. GUEsb and GUEl showed free-radical scavenging antioxidant activity, antihemolytic activity, and AAPH- as well as DOX-induced malondialdehyde content reduction in human erythrocytes. Based on its higher antioxidant potential, GUEsb was selected and subsequently showed intracellular ROS reduction without impairing the chemotherapeutic activity of DOX in K562 cells or inducing leukocyte cell death, but protected them against DOX-induced cell death. Yet, GUEsb did not show *in vivo* acute toxicity, and it prevented MDA generation in the cardiac tissue of DOX-treated mice, thus demonstrating its cardioprotective effect. Taken together, the results show that GUEsb and GUEl are natural alternatives to treat diseases associated with oxidative stress and that, in particular, GUEsb may play an adjuvant role in DOX chemotherapy.

1. Introduction

Oxidative stress is a condition of imbalance between the quantity of reactive species and the inefficient activity of the antioxidant protection system of an organism [1], and it is frequently associated with symptoms and diseases, including diabetes [2], inflammation [3], gastrointestinal [4] and cardiovascular [5] diseases, and anthracycline-induced cardiotoxicity [6].

Doxorubicin (DOX), an anthracycline antibiotic, is widely used to treat solid and hematological cancers [7]. In cancer cells, DOX causes DNA intercalation and disrupts the cellular repair process, thus increasing the production of reactive oxygen species (ROS) and triggering oxidative

stress [8]. Furthermore, studies indicate that DOX reduces the activity of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in the heart [9, 10]. The resulting reactive oxygen species cause cumulative and irreversible cardiomyocyte damage that can lead to apoptosis or even to dysfunction as well as cardiac failure. Therefore, cardiotoxicity is the main limitation of its clinical application [11].

Studies have shown that DOX-induced cardiotoxicity can be reduced by the coadministration of DOX and extracts from medicinal plants with antioxidant activity, including *Camellia sinensis* [12] and *Capparis spinosa* [13], and by their combination with phenolic compounds [10, 14, 15].

Guazuma ulmifolia Lam. (Malvaceae), commonly known as "mutamba" [16] or "guácimo" [17], is found in Latin American countries, including Brazil [18]. In traditional medicine, it is used as an infusion or decoction to treat inflammation [19], gastrointestinal diseases [20], and diabetes [21], which are associated with oxidative stress [2–4]. Pharmacological studies have confirmed the antidiabetic potential of stem bark and leaves [22, 23], the hypotensive and vasorelaxant effects of *G. ulmifolia* stem bark [24], and the antihypercholesterolemic [25] and gastroprotective [26] activity of *G. ulmifolia* leaves. Phytochemical studies of *G. ulmifolia* leaves, fruits [21], and stem bark [27] identified phenolic compounds that are reported in the literature for their antioxidant activity [28–30] and that may contribute to the pharmacological activities described above.

In this context, we aimed to analyze the chemical composition and antioxidant capacity of aqueous extracts from *G. ulmifolia* stem bark and leaves in human blood cells subjected to different oxidative agents. Furthermore, we assessed the acute toxicity effects of *G. ulmifolia* stem bark extracts and their ability to prevent DOX-induced cardiotoxicity *in vivo*.

2. Materials and Methods

2.1. Botanical Material and Extract Preparation. *G. ulmifolia* stem bark and leaves were collected with the permission of the Brazilian Biodiversity Authorization and Information System (Sistema de Autorização and Informação sobre Biodiversidade, SISBIO; no. 51092), in the municipality of Ivinhema/Mato Grosso do Sul state (MS) 22° 22′ 22.08″ south, 53° 54′ 57.58″ west. The identification of the species was confirmed by a botany specialist, and a voucher specimen was deposited in the herbarium (DDMS) of the Federal University of Grande Dourados (UFGD), Dourados, MS, under record number 5815. After collection, the stem bark and leaves were washed in running water and dried in a convection oven at 40°C for 5 days and at 36°C for 7 days, respectively. Then, both samples were ground in a Willey knife mill, sieved through a 10 mm mesh, and stored in polypropylene containers at −20°C.

To prepare the aqueous extract from *G. ulmifolia* steam bark (GUEsb), 100 g of dried stem bark powder was decocted in 1 L of water for 15 min and cooled for 5 min. Subsequently, centrifugation was performed at 5000 rpm for 15 min, and the supernatant was freeze-dried and stored in a freezer at −20°C. The aqueous extract from *G. ulmifolia* leaves (GUEl) was prepared by infusing 100 g of dried leaf powder in 1 L of water heated to 80°C for 15 min, followed by cooling for 5 min. Then, the infusion was centrifuged at 5000 rpm for 15 min, and the supernatant was centrifuged for another 5 min, freeze-dried, and stored in a freezer at −20°C. The total yields were 22% for GUEsb and 7.4% for GUEl.

2.2. Chemical Composition

2.2.1. Phytochemical Profile and Content. The phenolic content was determined using the method described by Meda et al. [31], with some modifications. Each extract was prepared at a final concentration of 100 $\mu g \cdot mL^{-1}$ in 80% ethanol. A 0.5 mL aliquot of that solution was added to 2.5 mL of Folin–Ciocalteu reagent (1 : 10) and incubated at room temperature for 5 min. Subsequently, 2.0 mL of 14% sodium carbonate was added, followed by stirring and incubation in the dark for 2 h. A standard curve was constructed using aliquots of ethanolic solution of gallic acid (1 $mg \cdot mL^{-1}$) with different concentrations (0.4–21.0 $\mu g \cdot mL^{-1}$). The absorbance was read at 760 nm against an 80% ethanol blank in a spectrophotometer (T70 UV/VIS Spectrometer, PG Instruments Ltd). The equation of the curve was derived by linear regression correlation between the gallic acid concentration and each absorbance reading, thus indirectly calculating the total phenolic content of each extract. Each sample was tested in triplicate, resulting in a mean value expressed as milligram equivalents of gallic acid per gram of extract (mg EGA·g^{-1} extract).

The total flavonoid contents of GUEsb and GUEl were determined as described by Liberio et al. [32], with some modifications. For such a purpose, each extract was prepared at a final concentration of 100 $\mu g \cdot mL^{-1}$ in methanol PA. A 0.5 mL aliquot of that solution was added to 4.5 mL of aluminum chloride (2%) and incubated at room temperature for 30 min. A standard curve was constructed using aliquots of the methanolic solution of quercetin (1 $mg \cdot mL^{-1}$) with different concentrations (0.4–21.0 $\mu g \cdot mL^{-1}$). The absorbance was read at 415 nm against a methanol blank. The equation of the curve was derived by linear regression correlation between the quercetin concentration and each absorbance reading, thus indirectly calculating the total flavonoid content of each extract. Each sample was tested in triplicate, resulting in a mean value expressed as milligram equivalents of quercetin per gram of extract (mg EQ·g^{-1} extract).

2.3. Antioxidant Potential

2.3.1. DPPH Free Radical Scavenging. The 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich) free radical scavenging activities of GUEsb and GUEl were assessed as described by Gupta and Gupta [33] with some modifications. A total of 200 μL of GUEsb or GUEl at different concentrations (1–2000 $\mu g \cdot mL^{-1}$) was added to 1800 μL of DPPH solution (0.11 mM) in 80% ethanol. The mixture was homogenized, incubated for 30 min at room temperature in the dark, and then read in a spectrophotometer at 517 nm against an 80% ethanol blank. Ascorbic acid (AA) and butylated hydroxytoluene (BHT) were used as standard antioxidants. Three independent experiments were performed in triplicate for each extract. The data were expressed as the concentration

necessary to inhibit 50% of the free radical (IC_{50}) and as the maximum activity (A_{max}). The percentage of inhibition in relation to the control (DPPH solution (0.11 mM)) was calculated using the following equation:

$$\%\text{DPPH inhibition} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right) \times 100. \quad (1)$$

2.3.2. Preparation of the Human Erythrocyte Suspension (10%). After approval of the study by the UFGD Research Ethics Committee under protocol number 073238/2016, peripheral blood samples (10 mL) were collected from healthy donors in tubes with sodium citrate and centrifuged at 2000 rpm for 5 min. Then, the plasma and leukocytes were removed, and the erythrocytes were subjected to three washes with saline (0.9% NaCl) at 2000 rpm, discarding the supernatant after each washing cycle. Subsequently, a solution of erythrocytes (10%) was prepared in 0.9% NaCl.

2.3.3. Hemolytic Activity of G. ulmifolia Extracts. The human erythrocyte suspension (10%) was incubated at 37°C for 30 min with different concentrations (25, 50, 100, 250, 500, and 1000 $\mu g \cdot mL^{-1}$) of GUEsb, GUEl, or AA (antioxidant standard). Then, 0.5 mL of 0.9% NaCl was added. After 240 min, the samples were centrifuged at 2000 rpm for 5 min, and the absorbance was read at 540 nm. Erythrocytes incubated with only 0.9% NaCl were used as controls [34].

2.3.4. Oxidative Hemolysis Inhibition in Human Erythrocytes Induced by 2,2′-Azobis(2-Amidinopropane) Dihydrochloride (AAPH) or DOX. The ability of GUEsb and GUEl to decrease AAPH-induced oxidative stress in human erythrocytes was assessed following the method described by Campos et al. [34] with some modifications. For such a purpose, the erythrocyte suspension was preincubated at 37°C for 30 min with different concentrations (25, 50, 100, 250, 500, and 1000 $\mu g \cdot mL^{-1}$) of GUEsb, GUEl, or AA (antioxidant standard). Then, 0.5 mL of AAPH (50 mM diluted in 0.9% NaCl) or DOX (300 $\mu g \cdot mL^{-1}$ diluted in 0.9% NaCl) solution was added. After 240 min, the samples were centrifuged at 2000 rpm for 10 min and read in a spectrophotometer at 540 nm. Total hemolysis was induced by incubation of the erythrocyte suspension in distilled water. Erythrocytes incubated with only AAPH or DOX were used as controls. Three independent experiments were conducted in duplicate for each extract. The percentage of hemolysis was calculated using the following formula:

$$\text{Hemolysis}\,(\%) = \left(\text{Abs}_{\text{sample}} \div \text{Abs}_{\text{total hemolysis}}\right) \times 100. \quad (2)$$

2.3.5. Malondialdehyde (MDA) Dosage. After 240 min of erythrocyte suspension incubation with the extract and the oxidative hemolysis inducer (AAPH or DOX), the samples were centrifuged, and a 0.5 mL aliquot of supernatant was added to a tube with 1 mL of 10 nM thiobarbituric acid (TBA, Merck, diluted in 75 mM monobasic potassium phosphate buffer, pH = 2.5), which was incubated in a water bath at 96°C for 45 min. Then, the samples were cooled in an ice bath for 15 min. Subsequently, each sample was added to 4 mL of butanol, homogenized, and centrifuged at 3000 rpm for 5 min, and the absorbance was read at 532 nm [34]. A total of 0.5 mL of 20 mM MDA and 1 mL of TBA solution was used as a control. Three independent experiments were performed in duplicate for each extract. The MDA content was expressed using the following formula:

$$\text{MDA}\left(\text{nmol} \cdot \text{mL}^{-1}\right) = \text{Abs}_{\text{sample}} \times \left(\frac{20 \times 220.32}{\text{Abs}_{\text{control}}}\right). \quad (3)$$

2.4. Cell Culture

2.4.1. Cell Culture Conditions. In this study, we used the chronic myeloid leukemia (K562) cell line cultured in RPMI 1640 media (Gibco, Brazil) supplemented with 10% fetal bovine serum (FBS), 100 $U \cdot mL^{-1}$ penicillin, and 100 $\mu g \cdot mL^{-1}$ streptomycin (Gibco, Brazil) at 37°C in an incubator with 5% CO_2.

2.4.2. Cellular Antioxidant Activity. GUEsb was selected for the other studies because it showed the best overall antioxidant activity. To assess the intracellular ROS scavenging capacity of GUEsb, we used the probe 2′,7′-dichlorofluorescin diacetate (DCFH-DA), according to the method by Wolfe and Liu [35] with some modifications. K562 cells (2×10^4 cells $well^{-1}$ in 96-well microplates) were incubated at 37°C with 20 μM DCFH-DA for 1 h, washed in Hank's balanced salt solution, and treated with different concentrations of GUEsb (3.12, 6.25, 12.5, and 25 $\mu g \cdot mL^{-1}$) as well as 500 μM H_2O_2. The fluorescence was measured for 1 h every 5 min at an excitation wavelength of 485 nm and at an emission wavelength of 520 nm using a microplate reader (DTX 800, Beckman, CA, USA). Cells with and without H_2O_2 in the presence of DCFH-DA were used as positive and negative controls, respectively. Quercetin was used as the antioxidant standard. Two independent experiments were performed in triplicate. The intracellular antioxidant activity was expressed as the percentage of inhibition of intracellular ROS produced by exposure to H_2O_2.

$$\text{Intracellular ROS level}\,(\%) = \left(\text{Abs}_{\text{sample}} \div \text{Abs}_{\text{Positive control}}\right) \times 100. \quad (4)$$

2.4.3. Cell Viability Assay. We assessed whether GUEsb affects the cytotoxic activity of DOX in K562 cells and whether it is able to decrease or inhibit DOX-induced human leukocyte death, according to the method by Mosmann [36], with some adaptations. The IC_{50} of DOX (0.5 $\mu g \cdot mL^{-1}$) in K562 cells was previously determined. To isolate leukocytes, total blood was diluted in 0.9% NaCl, transferred into a sterile tube with Ficoll–Paque at a 3 : 1 ratio, and centrifuged at 2000 rpm for 20 min. Then, the plasma was discarded, and the layer of leukocytes was washed 2x in 0.9% NaCl. After the preparation procedures, K562 cells (2×10^4 cells $well^{-1}$) or leukocytes (12×10^4 cells $well^{-1}$) were plated in 96-well microplates and treated with 50 μL of different concentrations of GUEsb (1.56, 3.12, 6.25, 12.5, and 25 $\mu g \cdot mL^{-1}$) in the presence or absence of 50 μL of DOX at its IC_{50} value (0.5 $\mu g \cdot mL^{-1}$, diluted in 0.9% NaCl) for 24, 48, and 72 h. DOX and culture medium were used as positive and negative controls, respectively. After the incubation period, the cells

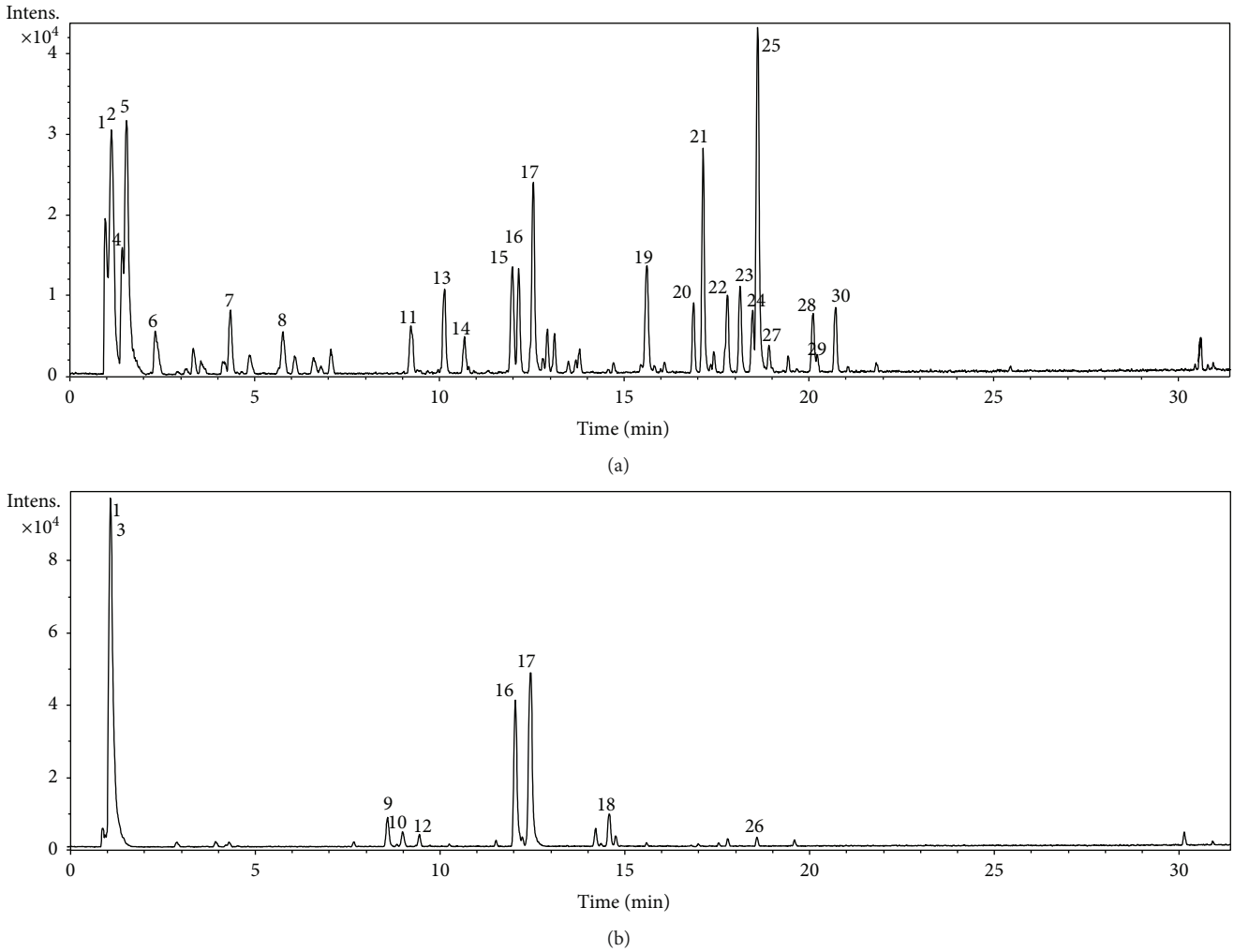

Figure 1: Total ion chromatogram in negative ion mode of aqueous extract from leaves (a) and stem bark (b) of *Guazuma ulmifolia*.

were centrifuged at 1500 rpm for 10 min and washed in phosphate-buffered saline (PBS), followed by the subsequent addition of 100 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (1 mg·mL^{-1} diluted in culture medium). After 240 min of incubation, the formazan crystals were resuspended in 100 μL of dimethylsulfoxide (DMSO), and the sample absorbance was read at 630 nm in a Thermoplate TP-READER. Three independent experiments were performed in triplicate. The cell viability was calculated using the following formula:

$$\text{Cell viability}\,(\%) = \left(\text{Abs}_{\text{sample}} \div \text{Abs}_{\text{Negative control}}\right) \times 100. \quad (5)$$

2.5. Animals

2.5.1. Animal Maintenance. This study was approved by the UFGD Ethics Committee on Animal Use, protocol number 29/2016, and was conducted in accordance with the ethical principles of animal experimentation adopted by the National Council for the Control of Animal Experimentation (Conselho Nacional de Controle de Experimentação Animal (CONCEA)). The animals were maintained under controlled temperature (22 ± 2°C) conditions and a 12 h light–dark cycle, and they were fed ad libitum.

2.5.2. Acute Toxicity Test in C57Bl/6 Mice. Acute toxicity was tested based on protocols from the Organization for Economic Cooperation and Development (OECD) Guideline 425 [37]. On the 1st day, one female C57Bl/6 mouse received 2000 mg·kg^{-1} of GUEsb orally (p.o.) after fasting for 8 h. The animal was regularly observed in the first 24 h. Subsequently, four other animals were subjected to the same procedure. The experimental procedure was repeated at a dose of 5000 mg·kg^{-1} towards defining the median lethal dose (LD_{50}) for the animals. Control animals ($n = 5$) received only water (orally). Then, the animals were observed once daily for 14 days. The body mass along with the food and water intake were recorded regularly. Hippocratic screening was performed to assess physiological and behavioral parameters (defecation, urination, exophthalmos, piloerection, tremors, hypersalivation, catatonia, tail erection, lacrimation, ataxia, pallor/hyperemia/cyanosis of the ears, paw licking, nose scratching, and tail biting). At the end of the study period, all animals were subjected to anesthesia with ketamine/xylazine and then euthanized. The organs (central nervous system, heart,

Table 1: Identification of the constituents from extracts of *G. ulmifolia* by LC-DAD-MS/MS.

Peak	RT (min)	Compound	UV (nm)	FM	Negative mode (*m*/*z*)		Positive mode (*m*/*z*)
					MS $[M-H]^-$	MS/MS	MS $[M+H]^+$
					341.1090	—	—
2	1.2	NI	—	$C_6H_{10}O_8$	209.0303	—	—
3	1.2	Quinic acid	—	$C_7H_{12}O_6$	191.0571	—	193.0717
4	1.4	Citric acid	—	$C_6H_8O_7$	191.0198	—	193.0343
5	1.5	Citric acid derivative	—	$C_6H_8O_7$	191.0195	—	193.0341
6	2.4	NI	—	$C_{14}H_{18}O_9$	329.0882	—	—
7	4.4	NI	—	$C_{14}H_{19}NO_7$	312.1078	—	336.1057^{Na}
8	5.8	NI	—	$C_{11}H_{12}N_2O_2$	203.0814		205.0970
9	8.6	Epigallocatechin*	278	$C_{15}H_{14}O_7$	305.0687	—	—
10	9.1	Catechin*	278	$C_{15}H_{14}O_6$	289.0735	—	—
11	9.2	PCY-PCY	278	$C_{30}H_{26}O_{12}$	577.1345	289	579.1501
12	9.5	PDE-PCY	280	$C_{30}H_{26}O_{13}$	593.1324		—
13	10.2	NI	280	$C_{15}H_{18}O_8$	325.0928		—
14	10.7	5-*O*-*E*-Caffeoylquinic acid*	299,325	$C_{16}H_{18}O_9$	353.0894	191	355.1030
15	11.9	NI	—	$C_{15}H_{19}NO_8$	340.1046	—	—
16	12.1	PCY-PCY	280	$C_{30}H_{26}O_{12}$	577.1357	407,3399,289,245,161	579.1497
17	12.5	Epicatechin*	280	$C_{15}H_{14}O_6$	289.0716	245,221,187,165	291.0880
18	14.6	PCY-PFI	280	$C_{30}H_{26}O_{11}$	561.1393	289,245,205,179,164	563.1580
19	15.6	NI	280	$C_{13}H_{14}N_2O_3$	245.0940	—	—
20	16.8	Di-*O*-deoxyhexosyl-hexosyl quercetin	270,355	$C_{33}H_{40}O_{20}$	755.2035	300,271,255,179	757.2218
21	17.1	Di-*O*-deoxyhexosyl-hexosyl quercetin	270,355	$C_{33}H_{40}O_{20}$	755.2044	300,271,255,179	757.2193
22	17.8	*O*-Deoxyhexosyl-hexosyl quercetin	265,350	$C_{27}H_{30}O_{16}$	609.1472	300,271,255,243	611.1628
23	18.1	*O*-Deoxyhexosyl-hexosyl quercetin	265,350	$C_{27}H_{30}O_{16}$	609.1467	300,271,255	611.1640
24	18.5	*O*-Hexosyl quercetin	270,350	$C_{21}H_{20}O_{12}$	463.0903	300,271,255,243	465.1036
25	18.6	*O*-Deoxyhexosyl-hexosyl quercetin	265,355	$C_{27}H_{30}O_{16}$	609.1473	300,271,255,179	611.1624
		Di-*O*-deoxyhexosyl-hexosyl kaempferol		$C_{33}H_{40}O_{19}$	739.2087	284	741.2210
26	18.6	PCY-PCY	280	$C_{30}H_{26}O_{12}$	577.1375	289	579.1508
27	18.9	*O*-Hexosyl quercetin	265,355	$C_{21}H_{20}O_{12}$	463.0893	300	465.1072
28	20.0	*O*-Pentosyl quercetin	265,350	$C_{20}H_{18}O_{11}$	433.0775	300,271,255,243	435.0918
29	20.2	*O*-Deoxyhexosyl quercetin	265,350	$C_{21}H_{20}O_{11}$	447.0937	300	449.1091
30	20.6	*O*-Deoxyhexosyl hexosyl luteolin	265,337	$C_{27}H_{30}O_{15}$	593.1498	284,255,227	595.16699

*Confirmed by authentic standard. NI: nonidentified; PDE: prodelphinidin; PFI: profisetinidin; PCY: procyanidin; RT: retention time; —: non-observed/detected means.

liver, spleen, lungs, and kidneys) were removed, weighed, and macroscopically analyzed. Blood was drawn for biochemical and hematological analysis.

2.5.3. DOX-Induced Cardiotoxicity in C57Bl/6 Mice. In vivo cardiotoxicity was induced by DOX, according to Momin et al. [9], with some modifications. Male C57Bl/6 mice of approximately 25 g were randomly distributed between groups ($n = 5$). The groups were treated as follows: (I) control (water, p.o.), (II) DOX (water, p.o.), and (III) DOX + GUEsb (200 mg GUEsb·kg^{-1} body mass, p.o.). From the 7th day, the animals received, in combination with GUEsb, the cumulative dose of DOX (totaling 24 mg·kg^{-1} diluted in 0.9% NaCl) by intraperitoneal injection (i.p.)) divided into six doses on alternate days (7th, 9th, 11th, 13th, 15th, and 17th). On the 18th day, the animals were anesthetized with ketamine/xylazine and euthanized, and the organs (heart, liver, and kidneys) were collected, weighed, and macroscopically evaluated. Furthermore, the following parameters were assessed: changes in body mass, food and water intake, relative organ mass, and MDA content of the heart.

(1) MDA Dosage. The MDA content of the heart was assessed according to the method adapted from Draper et al. [38]. The heart was triturated in 1.15% potassium chloride (KCl) and centrifuged at 3000 rpm for 10 min. Then, 0.5 mL of the supernatant was incubated with 1 mL of 10% trichloroacetic acid (TCA) and 1 mL of 20 nM TBA (diluted in 75 mM

monobasic potassium phosphate buffer, pH = 2.5) at 96°C for 45 min. After cooling, 3 mL of butanol was added. The mixture was homogenized and centrifuged at 3000 rpm for 5 min, and the absorbance was read at 532 nm. The control solution was 0.5 mL of 20 mM MDA and 1 mL of TBA. The MDA content was expressed using the following formula:

$$\text{MDA}\left(\text{nmol}\cdot\text{mL}^{-1}\right) = \text{Abs}_{\text{sample}} \times \left(\frac{20 \times 220.32}{\text{Abs}_{\text{control}}}\right). \quad (6)$$

2.6. Statistical Analysis. The results were expressed as the m ean ± standard error of the mean (SEM). The results were compared by analysis of variance (ANOVA) followed by the Student–Newman–Keuls posttest. Data were considered significant when $P < 0.05$. Statistical tests were performed using the statistical software GraphPad Prism 5.0.

3. Results

3.1. Chemical Composition. The chemical profile of *G. ulmifolia* extracts was identified based on UV, precise mass and tandem mass spectrometry (MS/MS) data compared with published data and the coinjection of standards (Figure 1). Compounds relative to the thirty chromatographic peaks were detected in the aqueous extracts of *G. ulmifolia* leaves and stem bark, and the main compounds identified were flavan-3-ol-derived flavonoids, including monomers and dimers, condensed tannins in GUEsb, and glycosylated flavonoids in GUEl (Table 1). The phenolic and flavonoid contents were 324.4 ± 4.1 and 240.0 ± 0.4 mg GAE·g^{-1} extract along with 12.9 ± 1.0 and 32.5 ± 1.3 mg EQ·g^{-1} extract in GUEsb and GUEl, respectively.

3.2. Antioxidant Capacity Assessment and Decreased Oxidative Stress

3.2.1. DPPH Free Radical Scavenging. GUEsb and GUEl showed high DPPH free radical scavenging activities, similar to that of the lipophilic antioxidant control BHT and lower than that of the hydrophilic antioxidant control AA, as shown by the IC_{50} and A_{max} values outlined in Table 2.

3.2.2. Determination of the Hemolytic Activity, AAPH-Induced Oxidative Hemolysis Inhibition, and MDA Dosage. GUEsb and GUEl showed no hemolytic activity at the concentrations tested, which was observed only at the highest concentration of ascorbic acid (Figure 2(a)). Then, the antioxidant potentials of GUEsb and GUEl against AAPH-induced hemolysis were analyzed. Both extracts decreased AAPH-induced hemolysis at 240 min of incubation more efficiently than AA; 25 and 1000 μg mL^{-1} GUEsb induced 16% and 83% protection, respectively, and GUEl induced 13% and 90% protection at 250 and 1000 μg mL^{-1}, respectively (Figure 2(b)).

Subsequent tests showed that both extracts decreased lipid peroxidation, as indicated by MDA levels lower than those of the control group (Figure 2(c)). GUEsb decreased MDA by 15% and 82% at 100 and 1000 μg mL^{-1}, and GUEl decreased MDA by 14% and 79% at 500 and 1000 μg mL^{-1}, respectively. Comparatively, AA decreased MDA production by 14% and 56% at 50 μg·mL^{-1} and 500 μg·mL^{-1}, respectively, and AA showed oxidant activity at the highest concentration tested (Figure 2(c)).

Table 2: Antioxidant activity of aqueous extracts from Guazuma ulmifolia stem bark (GUEsb) and leaves (GUEl).

	DPPH scavenging		
	IC_{50} [μg·mL^{-1}]	Maximum activity [μg·mL^{-1}]	(%)
AA	6.9 ± 1.0	25	96
BHT	21.5 ± 7.3	75	85
GUEsb	25.2 ± 5.1	100	91
GUEl	39.3 ± 8.8	100	84

IC50 and maximum activity of DPPH free radical scavenging of standard antioxidants and the aqueous extracts from *Guazuma ulmifolia* stem bark (GUEsb) and leaves (GUEl).

3.2.3. Inhibition of DOX-Induced Oxidative Hemolysis and MDA Production. When testing for protection against DOX-induced hemolysis, GUEsb and GUEl were able to protect human erythrocytes against oxidative hemolysis (Figure 3(a)) and MDA production (Figure 2(b)) after 240 min of incubation at all of the concentrations tested. The highest degrees of protection against hemolysis for GUEsb and GUEl were 54% and 48% at 25 μg·mL^{-1}, respectively. This protection was similar to that of the antioxidant standard AA, which was 62% at the same concentration (Figure 2(a)).

DOX-induced MDA production was also decreased by 38% and 36% upon incubation with 25 μg·mL^{-1} GUEsb and GUEl, respectively, compared with a 50% decrease caused by AA at the same concentration (Figure 3(b)).

3.2.4. Cellular Antioxidant Activity. We continued the studies only with GUEsb because it showed a higher overall antioxidant potential. K562 erythroleukemia cells subjected to H_2O_2-induced oxidative stress showed high intracellular ROS production, which was decreased by incubation with GUEsb at all of the concentrations tested, similarly to the activity of the antioxidant standard quercetin (Figure 4).

3.3. Cell Viability

3.3.1. Viability of K562 Erythroleukemia Cells Treated with GUEsb and Incubated with or without DOX. K562 cells incubated only with GUEsb showed decreased cell viability by 18% and 27% at 12.5 and 25 μg·mL^{-1}, respectively, at 24 h of incubation and by 18% at 25 μg·mL^{-1} and 48 h of incubation (Figure 5). K562 cells incubated with 0.5 μg·mL^{-1} DOX (the DOX IC_{50} of that cell line was previously determined) showed 42%, 72%, and 84% cell death at 24, 48, and 72 h of incubation, respectively. Combined treatment with DOX + GUEsb caused no change in the DOX-induced cell death profile, leading to similar cell death rates of 33%, 71%, and 84%, at the same incubation times, respectively.

3.3.2. Viability of Human Leukocytes Treated with GUEsb and Incubated with or without DOX. Leukocytes treated with

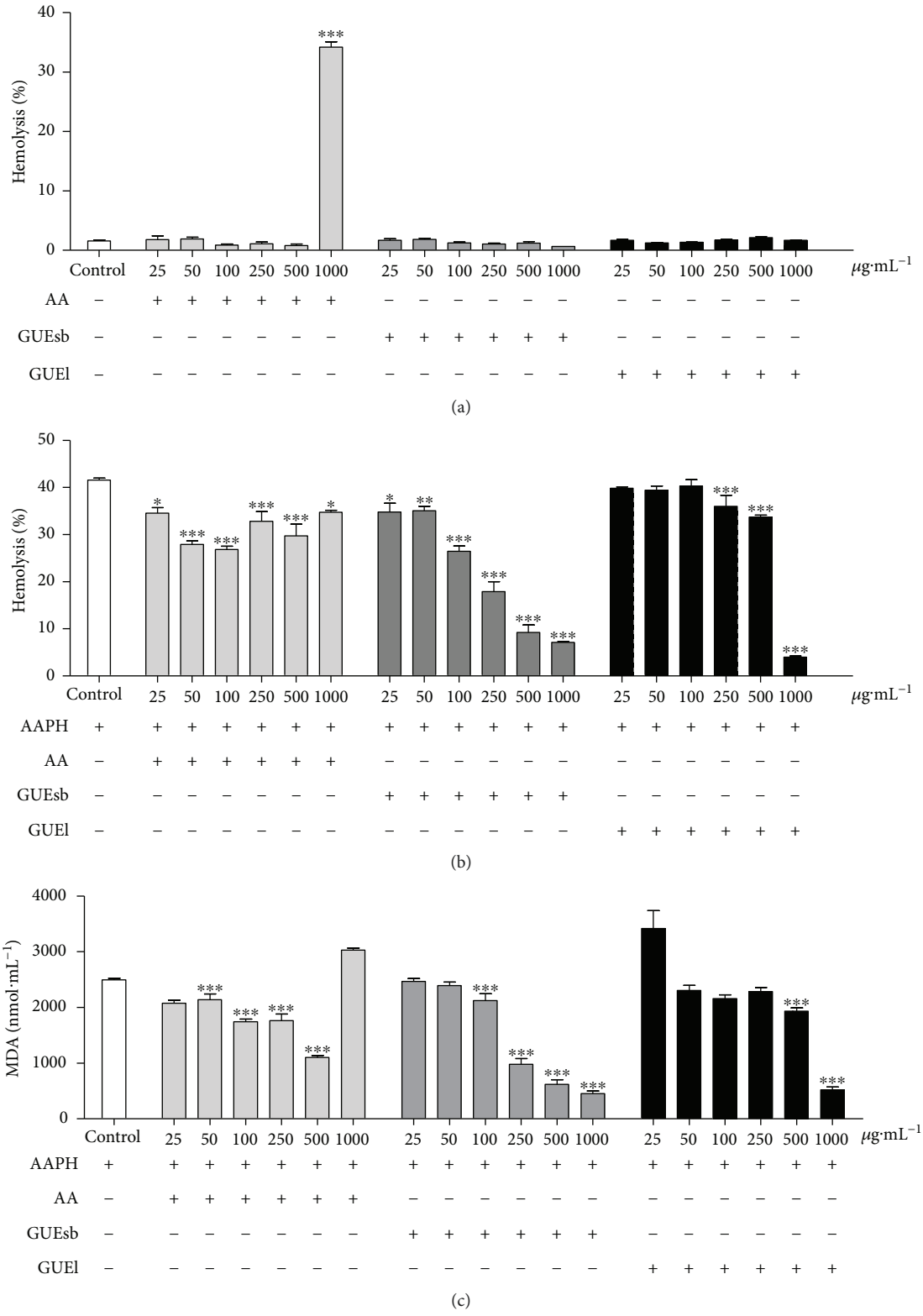

Figure 2: Hemolysis and MDA content of human erythrocytes. AA, GUEsb, or GUEl activity on (a) hemolysis, (b) hemolysis inhibition, and (c) malondialdehyde (MDA) content resulting from AAPH-induced lipid peroxidation. The data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the control (erythrocytes incubated with only AAPH). AA = ascorbic acid; GUEsb = aqueous extract from *G. ulmifolia* stem bark; GUEl = aqueous extract from *G. ulmifolia* leaves.

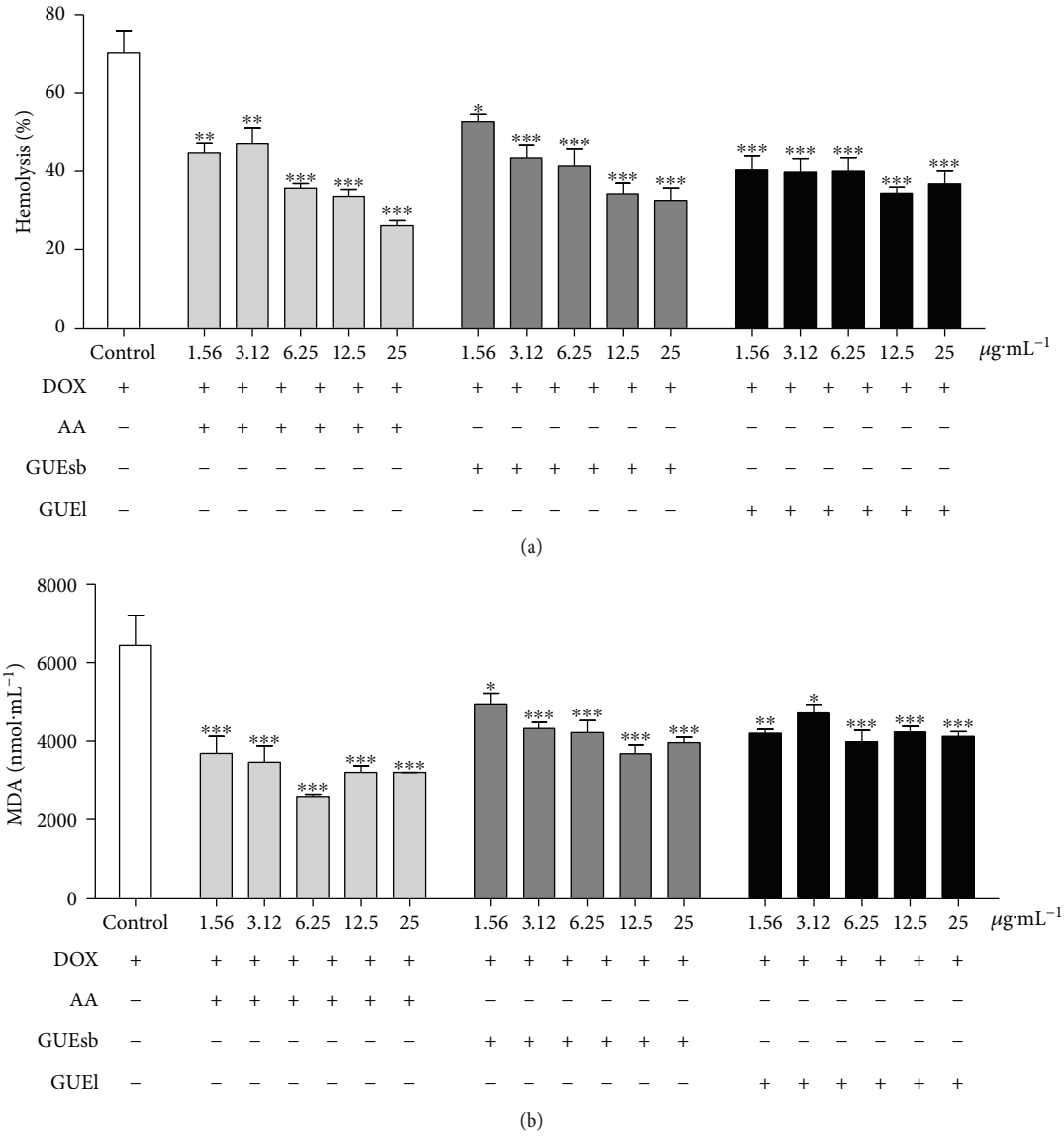

Figure 3: DOX-induced hemolysis and MDA content of human erythrocytes incubated for 240 min with AA, GUEsb, or GUEl (1.56–25 $\mu g \cdot mL^{-1}$). (a) Hemolysis inhibition at 240 min after adding DOX [300 $\mu g \cdot mL^{-1}$]. (b) Malondialdehyde (MDA) content resulting from DOX-induced lipid peroxidation [300 $\mu g \cdot mL^{-1}$] after 240 min. The data are expressed as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with the control (erythrocytes incubated with DOX only). AA = ascorbic acid; GUEsb = aqueous extract from *G. ulmifolia* stem bark; GUEl = aqueous extract from *G. ulmifolia* leaves.

only GUEsb showed no decrease in cell viability at any of the concentrations and times tested. However, leukocytes incubated with 0.5 $\mu g \cdot mL^{-1}$ DOX showed 19%, 20%, and 46% cell death after 24, 48, and 72 h of incubation, respectively. The combined treatment with 25 $\mu g \cdot mL^{-1}$ DOX + GUEsb was able to prevent DOX-induced cell death by 9% and 35% at 48 and 72 h, respectively (Figure 6).

3.4. Animals

3.4.1. Acute Toxicity Test in C57Bl/6 Mice. Female C57Bl/6 mice treated with 2000 and 5000 mg GUEsb·kg^{-1} body mass showed no signs of toxicity (Table 3), mortality, or physical and behavioral changes, except for an increase in creatinine at the highest dose, compared with the control group.

3.4.2. DOX-Induced Cardiotoxicity in C57Bl/6 Mice

(1) Body Mass, Food Intake, and Relative Organ Mass. Mice treated with DOX showed decreased body mass at the end of the treatment compared with the control group (Table 4). No changes were observed in the other parameters.

(2) Inhibition of DOX-Induced MDA Content in the Cardiac Tissue. Treatment with DOX increased the cardiac MDA content by approximately 48% compared with the control group. Combined treatment with DOX and GUEsb prevented this MDA production in the cardiac tissue and reduced the cardiac MDA content in the animals of the DOX + GUEsb group by 19% compared with the control group (Figure 7).

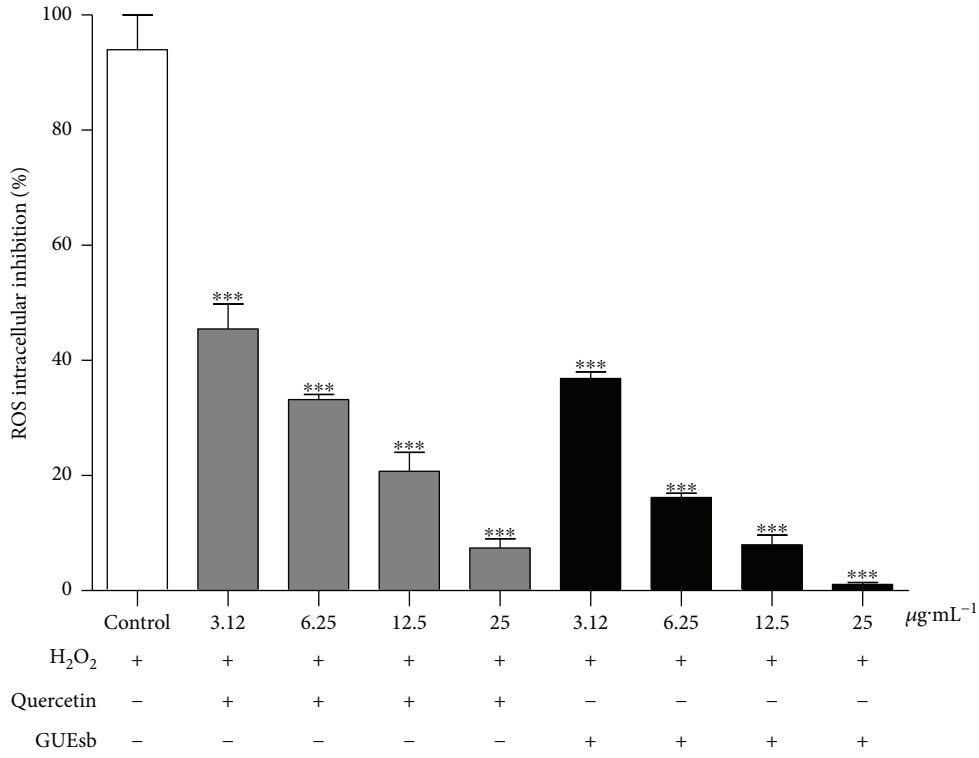

Figure 4: Cellular antioxidant activity. Inhibition of intracellular ROS production in K562 erythroleukemia cells incubated with DCFH-DA for 1 h, subsequently treated with quercetin or GUEsb (3.12, 6.25, 12.5, and 25 $\mu g \cdot mL^{-1}$) and immediately exposed to hydrogen peroxide (H_2O_2 500 μM). The data are expressed as the mean ± SEM. $^{***}P < 0.001$ compared with the control (cells incubated with DCF and exposed to H_2O_2). GUEsb = aqueous extract from *G. ulmifolia* stem bark.

4. Discussion

Medicinal plants are key targets in the search for therapeutic alternatives against oxidative stress because some phytochemicals, such as phenolic compounds, have antioxidant properties capable of maintaining the redox balance and protecting cells against damage caused by excess ROS [39]. In this study, several compounds, previously described in the literature, were identified in *G. ulmifolia* stem bark, such as phenolic acids, flavan-3-ol-derived flavonoids (monomers and dimers), and condensed tannins, including epicatechin, epigallocatechin, catechin, procyanidins, prodelphinidin–procyanidin, and procyanidin–profisetinidin [40, 41]. Phenolic acids and glycosylated flavonoids (with one, two, or three sugars), including chlorogenic acid, catechin, quercetin, and luteolin, were identified in leaf extracts [18, 42]. Furthermore, unpublished compounds were identified, namely, citric and quinic acids in *G. ulmifolia* stem bark and *O*-pentosyl quercetin, di-*O*-deoxyhesosyl-hesosyl quercetin, *O*-deoxyhexosyl hexosyl luteolin, and di-O-deoxyhexosyl-hexosyl kaempferol in *G. ulmifolia* leaves.

The quantity of phenolic compounds can directly affect the biological potential of natural products [43], including the antioxidant activity of medicinal plants [43–45]. In this study, a high phenolic content was found in both extracts, and GUEsb showed a higher phenolic content than GUEl and one similar to that found by Feltrin et al. [27] in 70% hydroethanolic extract from *G. ulmifolia* stem bark. GUEsb showed a higher DPPH radical scavenging activity than GUEl. The highest flavonoid content was found in GUEl, which was even higher than that found by Morais et al. [42] in the ethanolic extract from *G. ulmifolia* leaves, and our GUEl showed a higher free-radical scavenging capacity than that found in the previous study. When compared with antioxidant standards, both extracts were inferior to AA and similar to the antioxidant standard BHT, an isolated synthetic compound widely used in the cosmetic, pharmaceutical, and food industries [46], which has been associated with the development of cardiac diseases and carcinogenesis [47, 48], thus indicating the need for new substitutes, particularly natural compounds. Taken together, this evidence supports the traditional medicine [19] procedure of aqueous extraction as an efficient method to isolate bioactive compounds present in *G. ulmifolia*.

To best understand the biological potential of the *G. ulmifolia* extracts, we used human blood cells subjected to oxidative stress induced by different oxidant agents. Initially, we used AAPH, a water-soluble azo compound that decomposes at 37°C generating peroxyl radicals (ROO) [49] responsible for oxidizing erythrocyte membrane lipids and proteins [50]. Azo compound-derived ROO and those formed physiologically and pathologically *in vivo* react with biomolecules similarly, facilitating the study of the oxidation kinetics of biological molecules and their possible protection [51]. Both extracts, GUEsb and GUEl, decreased human erythrocyte lysis and the content of MDA produced, even more

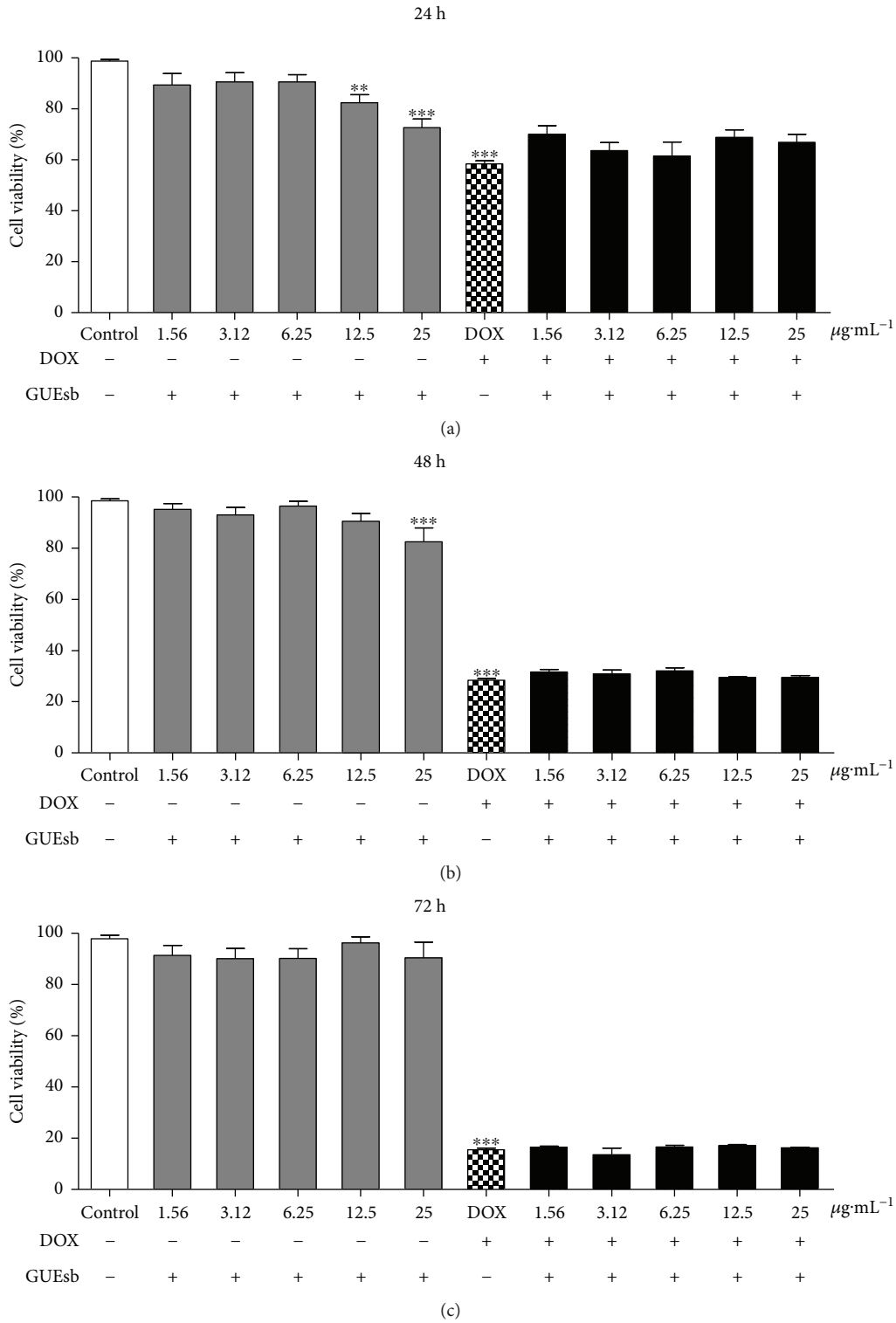

FIGURE 5: DOX-induced cytotoxicity in K562 erythroleukemia cells treated with GUEsb (1.56–25 $\mu g \cdot mL^{-1}$) for 24, 48, and 72 h. Viability of K562 cells treated with GUEsb and incubated with or without DOX (0.5 $\mu g \cdot mL^{-1}$) for (a) 24, (b) 48, and (c) 72 h. The data are expressed as the mean ± SEM. Only the cells treated with GUEsb were compared with the control (K562 cells incubated with culture media only), and significant differences were identified when $^{**}P < 0.01$ and $^{***}P < 0.001$. The cells treated with DOX + GUEsb were compared with DOX (K562 cells incubated with 0.5 $\mu g \cdot mL^{-1}$ DOX). GUEsb = aqueous extract from *G. ulmifolia* stem bark.

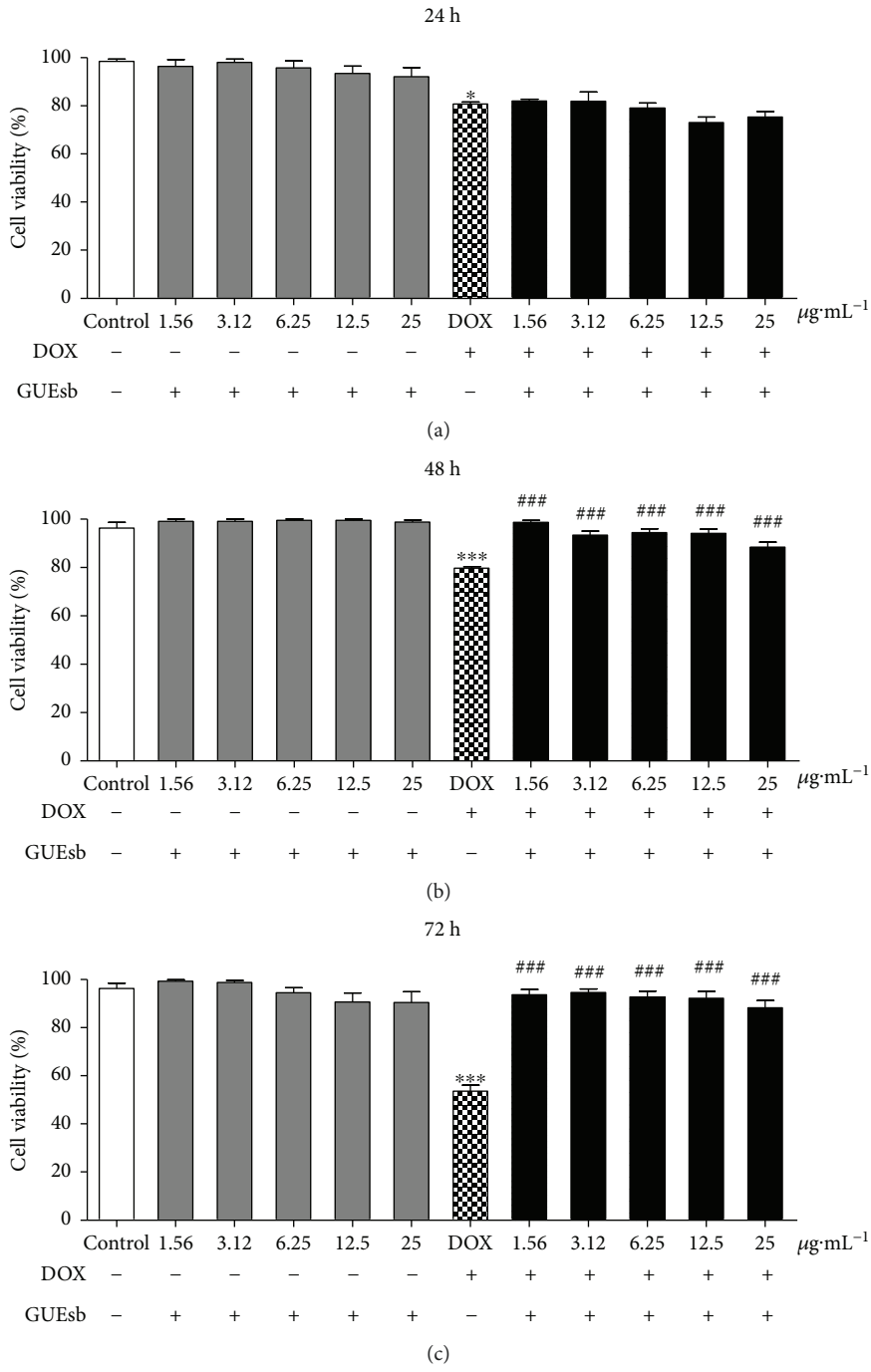

Figure 6: DOX-induced cytotoxicity in human leukocytes treated with GUEsb (1.56–25 $\mu g \cdot mL^{-1}$) for 24, 48, and 72 h. Viability of human leukocytes treated with GUEsb and incubated with or without DOX (0.5 $\mu g \cdot mL^{-1}$) for (a) 24, (b) 48, and (c) 72 h. The data are expressed as the mean ± SEM. Only the cells treated with GUEsb were compared with the control (human leukocytes incubated with culture media only), and significant differences were identified when $^{*}P < 0.05$ and $^{***}P < 0.001$. The cells treated with DOX + GUEsb were compared with DOX (human leukocytes incubated with 0.5 $\mu g \cdot mL^{-1}$ DOX), and significant differences were identified when $^{###}P < 0.001$. GUEsb = aqueous extract from *G. ulmifolia* stem bark.

efficiently than the antioxidant standard AA, which has a lower protective activity and even behaved as an oxidant at the highest concentration tested, which may be related to Fenton's reaction. In this process, ascorbate reduces metal ions, thereby generating intermediate radicals [52, 53]. The protective effect of GUEsb against oxidative hemolysis was even stronger than those of other extracts in the same biological model [43, 54].

Erythrocytes were also exposed to another oxidant agent, the chemotherapeutic doxorubicin, which is widely used to treat several types of cancer. However, the oxidative stress generated by this drug is indicated as one of the main

Table 3: Body mass evolution, food and water intake, hematological parameters, biochemical parameters, and relative mass of the organs of female mice treated with single doses of GUEsb.

Parameters	Control	GUEsb	
		2000 mg·kg^{-1}	5000 mg·kg^{-1}
Evolution body weight (%)	0.00 ± 1.83	−0.40 ± 1.67	−2.20 ± 1.02
Food intake (g·day^{-1})	14.30 ± 1.10	16.42 ± 1.50	17.30 ± 1.41
Water intake (mL^{-1}·day)	26.20 ± 2.43	28.00 ± 1.50	28.85 ± 1.64
WBC (10^3·μL^{-1})	3.84 ± 0.87	2.80 ± 0.90	3.60 ± 0.60
RBC (10^6·μL^{-1})	10.30 ± 0.30	9.70 ± 0.33	10.00 ± 0.57
HGB (g·dL^{-1})	13.50 ± 0.41	12.84 ± 0.44	13.52 ± 0.60
HCT (%)	53.10 ± 1.73	49.42 ± 1.90	53.20 ± 2.22
MCV (fL)	52.40 ± 1.10	51.02 ± 0.50	53.30 ± 0.50
MCH (pg)	13.30 ± 0.30	13.30 ± 0.20	13.54 ± 0.10
MCHC (g·dL^{-1})	25.40 ± 0.20	26.12 ± 0.31	25.40 ± 0.30
PLT (10^3·μL^{-1})	968.8 ± 114.9	1204.2 ± 49.1	1049.2 ± 107.9
Neutrophil (10^3·μL^{-1})	0.53 ± 0.12	0.33 ± 0.14	0.37 ± 0.08
Linfocyte (10^3·μL^{-1})	3.30 ± 0.80	2.45 ± 0.80	3.20 ± 0.50
AST (U·L^{-1})	63.50 ± 4.80	59.70 ± 1.10	68.40 ± 15.60
ALT (U·L^{-1})	35.70 ± 4.70	30.70 ± 1.91	30.20 ± 3.21
Urea (mg·dL^{-1})	48.52 ± 3.90	52.22 ± 1.84	53.70 ± 2.21
Creatinine (mg·dL^{-1})	0.20 ± 0.01^a	0.20 ± 0.01^a	0.30 ± 0.02^b
CNS (g·100^{-1} of body weight)	0.41 ± 0.06	0.42 ± 0.05	0.55 ± 0.06
Heart (g·100^{-1} of body weight)	0.44 ± 0.03	0.44 ± 0.02	0.44 ± 0.06
Liver (g·100^{-1} of body weight)	4.17 ± 0.13	3.91 ± 0.12	4.17 ± 0.09
Spleen (g·100^{-1} of body weight)	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.01
Lung (g·100^{-1} of body weight)	0.55 ± 0.06	0.52 ± 0.06	0.60 ± 0.04
Kidney (g·100^{-1} of body weight)	1.01 ± 0.02	0.94 ± 0.030	1.10 ± 0.02

CNS = central nervous system; WBC = white blood cells; RBC = erythrocytes; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet; AST = aspartate aminotransferase; ALT = alanine aminotransferase. Data were expressed as mean ± SEM. GUEsb = aqueous extract of *G. ulmifolia* stem bark. Different superscript letters indicate statistically significant differences.

Table 4: Evolution of body mass, food and water consumption, and relative mass of organs of C57Bl/6 mice induced to oxidative stress with DOX.

Parameters	Control	DOX	DOX + GUEsb
Evolution body weight (%)	1.0 ± 0.85^a	−5.0 ± 1.17^b	−6.6 ± 3.02^b
Food intake (g·day^{-1})	37.1 ± 4.74	28.1 ± 4.11	27.8 ± 3.46
Water intake (mL^{-1}·day)	25.9 ± 0.86	22.0 ± 1.85	21.9 ± 1.67
Heart (g·100^{-1} of body weight)	0.48 ± 0.02	0.55 ± 0.05	0.47 ± 0.01
Liver (g·100^{-1} of body weight)	4.36 ± 0.13	4.94 ± 0.16	4.87 ± 0.19
Kidney (g·100^{-1} of body weight)	1.08 ± 0.07	1.05 ± 0.02	1.02 ± 0.01

The data are expressed as the mean ± SEM ($n = 5$). Different letters signify statistical differences at $P < 0.05$. GUEsb = aqueous extract from *G. ulmifolia* stem bark.

inducers of cardiotoxicity leading to the development of severe heart diseases [11]. Approximately 30% of patients subjected to chemotherapy with DOX develop cardiac dysfunction [55]. In this context, efforts have been directed towards searching for antioxidant compounds, such as dexrazoxane, which are able to prevent or attenuate the toxicity caused by this drug, and this topic is one of the focuses of discussion of the International Cardioncology Society [56].

In this study, the oxidative stress signs in human erythrocytes exposed to DOX, including increased hemolysis and MDA, were reduced by the combined use of GUEsb or GUEl with DOX. The antioxidant activity of *G. ulmifolia* extracts against AAPH- and DOX-induced oxidative stress may be partly attributed to the presence of phenolic compounds because they are able to chelate metal ions and inhibit Fenton's reaction, particularly flavonoids such as quercetin

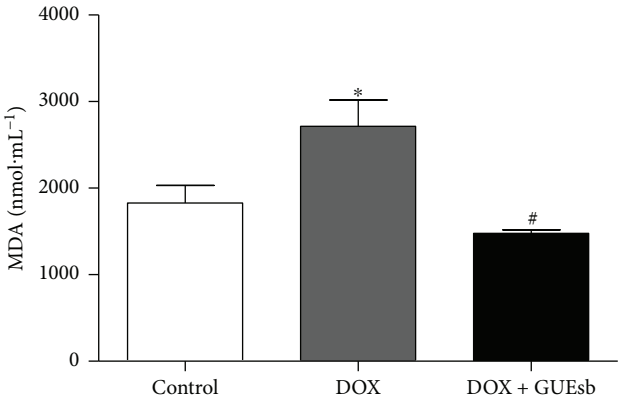

Figure 7: DOX-induced cardiotoxicity in C57Bl/6 mice. MDA content of the control (water), DOX (water + DOX cumulative dose of 24 mg·kg^{-1}), and DOX + GUEsb (GUEsb 200 mg·kg^{-1} + DOX cumulative dose of 24 mg·kg^{-1}) mouse heart tissue after 18 days. The data are expressed as the mean ± SEM ($n = 5$). $^{*}P < 0.05$ compared with control and $^{\#}P < 0.05$ compared with DOX. GUEsb = aqueous extract from *G. ulmifolia* stem bark.

present in leaves and catechin present in the stem bark [57]. Moreover, the presence of aromatic rings allows H^+ and electron donation, preventing the formation of ROS, such as OH^+ and ROO [58], which explains the decrease in lipid peroxidation.

The phytochemical composition and the previous results indicated a higher antioxidant potential of GUEsb, which was selected for the other tests. Subsequently, we confirmed, using a fluorescent probe, that GUEsb induced intracellular ROS scavenging in a K562 erythroleukemia line exposed to the oxidant agent H_2O_2 as efficiently as the control quercetin. This detoxification role may be played by both catechin [59] and quinic acid [60] or even by the synergism between them, resulting in increased CAT activity, which is the enzyme responsible for converting H_2O_2 into water molecules.

Antioxidants can attenuate oxidative damage and become promising strategies in chemotherapy, but the anticancer activity of the drug must not be impaired [61]. Although GUEsb caused a slight increase in cell death at the initial treatment times, when combined with DOX in K562 erythroleukemia cells, it had no effect on DOX-induced cell death. The ability to attenuate oxidative stress without affecting the cytotoxic activity of DOX is a key characteristic for the application of GUEsb as an adjuvant and may be related to the presence of flavonoids, which can reduce the negative effects of DOX without affecting the activity of the drug [62].

In addition to oxidative stress, DOX impairs leukocyte formation, causing leucopenia [63], most likely linked to the high content of polyunsaturated fatty acids in the membrane of those cells, which renders them highly sensitive to ROS [64]. GUEsb has immunoprotective effects on this condition, preventing DOX-induced death. This activity may be related to the antioxidant properties of the phenolic compounds of GUEsb. Furthermore, it should be noted that GUEsb contains procyanidins, which are associated with improved leucopenia symptoms in animals subjected to chemotherapy-induced immunosuppression [65].

However, cardiotoxicity is still the major limitation for the clinical application of DOX [11, 62]. The mechanism of anthracycline-induced cardiotoxicity is unclear, although the most commonly discussed hypotheses are DNA damage by increased production of reactive species and mitochondrial dysfunction caused by inhibition of topoisomerases II, which are the mechanisms of action of DOX in cancer cells [8]. Some factors increase the heart susceptibility to DOX-induced toxicity, such as high oxidative metabolism, decreased antioxidant enzymes [66], and, especially, the high DOX affinity for cardiolipin, a phospholipid essential to the mitochondrial structure and function as well as the energy metabolism of cardiomyocytes [67]. The formation of a strong DOX–cardiolipin complex results in DOX retention within the mitochondrial membrane, allowing continuous redox cycles, thereby causing oxidative damage [6]. However, inhibition of topoisomerases II is indicated as the main mediator of DOX-induced cardiotoxicity, since this drug promotes intercalation into the base pairs and topoisomerase-IIα inhibition-mediated disruption of DNA repair and mitochondrial dysfunction as a consequence topoisomerase-IIβ inhibition-mediated peroxisome proliferator-activated receptor (PPAR) suppression, leading to cell death [8, 68, 69]. Consequently, both mechanisms culminate in the leads to the loss of functional myocytes and to irreversible cardiac tissue damage because these cells do not regenerate [68].

DOX coadministration with natural antioxidants, including isolated phenolic compounds [10, 14, 15], and extracts from medicinal plants, such as *Ixora coccinea* Linn [9], *Camellia sinensis* [12], *Capparis spinosa* [13], *Vaccinium macrocarpon* [70], and *Melissa officinalis* [71], aims at finding alternative therapies to mitigate cardiac damage. In this study, GUEsb-induced cardioprotection in animals treated with DOX was stronger than that of other plants, which, even at higher doses [12, 71], only mitigated DOX-induced cardiotoxicity in rats. GUEsb was able to prevent MDA production in the cardiac tissue of animals treated with DOX. Previous studies indicate that procyanidin [72] and catechin [59], compounds also found in GUEsb, are able to reduce DOX-induced lipid peroxidation. Moreover, catechins have chelating properties and modulate the activity of antioxidant enzymes (SOD, CAT, and glutathione peroxidase) [59]. Accordingly, GUEsb may have been able to prevent DOX complexation with iron ions and to enhance ROS detoxification in the cardiac tissue. Our results suggest that the cardioprotective effects of GUEsb result from oxidative stress suppression mediated by its phytochemical constituents, which was corroborated by direct ROS scavenging and decreased lipid peroxidation in human erythrocytes and mice cardiomyocytes.

Toxicity data indicate that GUEsb is safe for consumption, based on acute lethality tests, physical and behavioral changes, and biochemical and hematological parameters assessing the toxic effects of several plant extracts in animal models [73–76]. GUEsb induced no physical or behavioral changes in the animals tested nor any changes in the food

and water intake, body mass, relative organ mass, or biochemical and hematological parameters. Therefore, GUEsb is safe for consumption.

5. Conclusion

Taken together, our results show that GUEsb and GUEl have antioxidant activity and are able to decrease oxidative stress in human blood cells, including DOX-induced oxidative stress, indicating that both extracts are possible, natural alternatives to treat diseases associated with oxidative stress. Furthermore, GUEsb showed no effect on the cytotoxicity of the drug or toxicity and was able to suppress DOX-induced cardiotoxicity.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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5. CONCLUSÃO

Em conjunto nossos resultados mostram que os extratos aquosos da casca e da folha de *G. ulmifolia* têm atividade antioxidante e são capazes de reduzir o estresse oxidativo em células sanguíneas humanas, inclusive induzido pelo quimioterápico DOX, indicando que ambos os extratos são possíveis alternativas naturais para o tratamento de doenças associadas ao estresse oxidativo. Adicionalmente, o EcGU não interferiu na ação anticâncer da droga, não foi tóxico e foi capaz de suprimir a cardiotoxicidade induzida por DOX, o que indica que este extrato pode desempenhar papel adjuvante na quimioterapia com DOX.

ANEXO I - Informações sobre a revista

O artigo científico descrito nesta dissertação foi publicado em uma edição especial da revista **Oxidative Medicine and Cellular Longevity**.

As normas para submissão encontram-se disponíveis em:< http://www.hindawi.com/journals/omcl/guidelines/>.

Fator de Impacto: 4,93

AIMS AND SCOPE

Oxidative Medicine and Cellular Longevity is a unique peer-reviewed journal that publishes original research and review articles dealing with the cellular and molecular mechanisms of oxidative stress in the nervous system and related organ systems in relation to aging, immune function, vascular biology, metabolism, cellular survival and cellular longevity. Oxidative stress impacts almost all acute and chronic progressive disorders and on a cellular basis is intimately linked to aging, cardiovascular disease, cancer, immune function, metabolism and neurodegeneration. The journal fills a significant void in today's scientific literature and serves as an international forum for the scientific community worldwide to translate pioneering "bench to bedside" research into clinical strategies.

FUNDAÇÃO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS/UFGD-MS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Caracterização do potencial farmacológico de Guazuma ulmifolia Lam.
Pesquisador: Jéssica Maurino dos Santos
Área Temática:
Versão: 2
CAAE: 58154816.3.0000.5160
Instituição Proponente: Faculdade de Ciências Biológicas e Ambientais
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.816.122

Apresentação do Projeto:
O presente projeto tem por objetivo avaliar o potencial farmacológico de Guazuma ulmifolia Lam. Para tanto, folhas e casca de G. ulmifolia serão coletadas, secas, trituradas e utilizadas para a preparação dos extratos aquosos, os quais serão avaliados quanto a seu potencial antioxidante e em síndrome metabólica. A atividade antioxidante será avaliada in vitro pelos ensaios de captura do radical livre 2,2', proteção contra hemólise induzida por 2,2' e conteúdo de malondialdeído gerado em eritrócitos induzidos com AAPH e, in vivo, pelo nível de MDA nos tecidos de animais com SM induzida por dieta tratados com os extratos. Quanto ao papel dos extratos em SM, serão avaliados animais tratados concomitantemente com a dieta para indução de SM e os extratos, e animais com SM induzida por dieta tratados com os extratos por 30 dias. Em ambos os grupos

Objetivo da Pesquisa:
Os objetivos da pesquisa estão descritos da seguinte maneira:
Objetivo Primário: Avaliar a atividade antioxidante de Guazuma ulmifolia Lam. e sua ação em modelo animal de síndrome metabólica (SM);
Objetivo Secundário: Serão avaliados os extratos aquosos de folhas e cascas de G. ulmifolia quanto a:- atividade antioxidante, in vitro e em eritrocitos humanos; - ação na
prevenção e tratamento da SM.- toxicidade.

Endereço: Rua Melvin Jones, 940
Bairro: Jardim América **CEP:** 79.803-010
UF: MS **Município:** DOURADOS
Telefone: (67)3410-2853 **E-mail:** cep@ufgd.edu.br

Avaliação dos Riscos e Benefícios:

Os riscos previsto no projeto estão relacionados ao procedimento de coleta de sangue venoso, a saber: leve mal estar, hipotensão leve e transitória, hematoma na área de coleta, desconforto pela pressão do “garrote” no braço e leve ardência pela penetração da agulha. Evidentemente, existem riscos - não mencionados - quanto à divulgação indevida de dados pessoais dos participantes, manuseio inadequado de animais e outros para os quais a pesquisadora deve estar atenta.

Quanto aos benefícios, arrolam-se:

1) Acadêmicos: os resultados deverão originar trabalhos de mestrado e iniciação científica que serão publicados como artigos científicos em revistas internacionais e os resultados parciais serão apresentados em eventos científicos e tecnológicos, além de contribuir na formação de um mestre em Biologia geral/Bioprospecção.

2) Saúde pública: investigação e compreensão do potencial farmacológico da Guazuma ulmifolia utilizada para fins terapêuticos pela população, de modo a contribuir para o desenvolvimento de dados que subsidiem a elaboração de futuros produtos farmacológicos, tornando-se uma alternativa terapêutica para redução do estresse oxidativo, condição envolvidas nos processos de doenças como dislipidemia, diabetes, câncer, hipertensão,

entre outras.

3) Conservação da biodiversidade:desenvolvimento de informações que contribuam para a valoração da espécie e conservação da biodiversidade.

Comentários e Considerações sobre a Pesquisa:

A pesquisa é relevante e o propósito de explorar potenciais farmacológicos de plantas da flora brasileira pode trazer contribuições ao tratamento de determinadas doenças.

Considerações sobre os Termos de apresentação obrigatória:

Os termos estão corretamente apresentados.

Recomendações:

Não há recomendações a fazer.

Conclusões ou Pendências e Lista de Inadequações:

As recomendações constantes do parecer anterior foram cumpridas. O projeto pode ser aprovado.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

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Continuação do Parecer: 1.816.122

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_754228.pdf	18/10/2016 23:18:33		Aceito
Projeto Detalhado / Brochura Investigador	Formulariopesquisacompleto.doc	18/10/2016 23:13:51	Jéssica Maurino dos Santos	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	18/10/2016 22:56:34	Jéssica Maurino dos Santos	Aceito
Outros	ParecerCEUA292016.pdf	18/10/2016 22:45:40	Jéssica Maurino dos Santos	Aceito
Cronograma	cronograma.doc	27/07/2016 20:19:36	Jéssica Maurino dos Santos	Aceito
Folha de Rosto	folhaderosto.pdf	27/07/2016 20:01:48	Jéssica Maurino dos Santos	Aceito
Declaração de Pesquisadores	declaracaoresultadopesquisa.docx	25/07/2016 21:21:59	Jéssica Maurino dos Santos	Aceito
Orçamento	Orcamento.doc	24/07/2016 22:25:30	Jéssica Maurino dos Santos	Aceito
Outros	resolucao.jpg	05/07/2016 22:41:21	Jéssica Maurino dos Santos	Aceito
Declaração de Instituição e Infraestrutura	termo_compromisso.jpg	05/07/2016 22:36:35	Jéssica Maurino dos Santos	Aceito
Declaração de Instituição e Infraestrutura	infraestrutura.jpg	05/07/2016 22:32:45	Jéssica Maurino dos Santos	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

DOURADOS, 09 de Novembro de 2016

Assinado por:
Leonardo Ribeiro Martins
(Coordenador)

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MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS
PRÓ-REITORIA DE ENSINO DE PÓS-GRADUAÇÃO E PESQUISA

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

Dourados-MS, 8 de setembro de 2016.

CERTIFICADO

Certificamos que a proposta intitulada **"Caracterização do potencial farmacológico de** *Guazuma ulmifolia* Lam**",** registrada sob o protocolo de nº 29/2016, sob a responsabilidade de *Kely de Picoli Souza e Jéssica Maurino dos Santos* – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo *Chordata, subfilo Vertebrata* (exceto o homem), para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto n° 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela Comissão de Ética no Uso de Animais (CEUA/UFGD) da Universidade Federal da Grande Dourados, em reunião de 01/07/2016.

Finalidade	*(X) Ensino ( ) Pesquisa Científica*
Vigência da autorização	*01/10/2016 a 30/03/2017*
Espécie/linhagem/raça	*Mus Musculus/ C57Bl/6*
Nº de animais	180
Peso/idade	20 g / 50 dias
Sexo	160machos e 20 fêmeas
Origem	Biotério Central da UFGD

Melissa Negrão Sepulvida

Melissa Negrão Sepulvida
Coordenadora CEUA