UNIVERSIDADE FEDERAL DA GRANDE DOURADOS
FACULDADE DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

UNIVERSIDADE DE COIMBRA
FACULDADE DE MEDICINA DA UNIVERSIDADE DE COIMBRA
DOUTORAMENTO EM CIÊNCIAS DA SAÚDE
RAMO DAS CIÊNCIAS BIOMÉDICAS

Avaliação do potencial farmacológico do extrato aquoso das folhas de *Acrocomia aculeata* (Jacq.) Lodd ex. Mart.

TAMAEH MONTEIRO ALFREDO

Dourados - MS
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Área do CNPq: Ciências da Saúde

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ATA DA DEFESA DE TESE DE DOUTORADO APRESENTADA POR **TAMAEH MONTEIRO ALFREDO** ALUNA DO PROGRAMA DE PÓS-GRADUAÇÃO *STRICTO SENSU* EM CIÊNCIAS DA SAÚDE, ÁREA DE CONCENTRAÇÃO "FARMACOLOGIA".

Aos vinte e nove dias do mês de outubro de dois mil e vinte e um, às 09 horas, em sessão pública, realizou-se por videoconferência a Defesa de Tese de Doutorado, conforme acordo de cotutela com a Universidade de Coimbra – UC, intitulada **"Avaliação do potencial farmacológico do extrato aquoso das folhas de *Acrocomia aculeata* (Jacq.) Lodd ex. Mart."** apresentada pela aluna **Tamaeh Monteiro Alfredo**, do Programa de Pós-Graduação em Ciências da Saúde, à Banca Examinadora constituída pelos membros: Prof.ª Dr.ª Kely de Picoli Souza - PPGCS/UFGD (presidente-orientadora), Dr. Paulo Nuno Centeio Matafome (orientador pela Universidade de Coimbra)/FMUC, Dr. Nelson Luis de Campos Domingues - Programa de Pós Graduação em Química na UFGD e no Programa de Pós Graduação em Biotecnologia e Conservação da Biodiversidade da Rede Pró-Centro Oeste/UFGD (membro titular externo), Dr. Caio Fernando Ramalho de Oliveira - Pós Doc na UFMS, Faculdade de Medicina UFMS (membro titular externo), Dr. Edson Lucas dos Santos - PPGCS/UFGD (membro titular interno), Dr.ª Ana Margarida Coelho Abrantes/FMUC (membro titular interno da FMUC) e Dr. Flávio Nelson Fernandes Reis/FMUC (membro titular interno da FMUC). Iniciados os trabalhos, a presidência deu a conhecer à candidata e aos integrantes da Banca as normas a serem observadas na apresentação da Tese. Após a candidata ter apresentado a sua explanação, os componentes da Banca Examinadora fizeram suas arguições. Terminada a Defesa, a Banca Examinadora, em sessão secreta, passou aos trabalhos de julgamento, tendo sido a candidata considerada RECUSADO ( ), APROVADA ( ), APROVADO COM DISTINÇÃO ( ), APROVADO COM DISTINÇÃO E LOUVOR (**X**), fazendo *jus* ao título de **DOUTORA EM CIÊNCIAS DA SAÚDE**. **A presidente da banca abaixo-assinado atesta que todos os membros participaram de forma remota[1] desta defesa de tese, considerando a candidata**

1 Participação remota dos membros da banca conforme § 3º do Art. 1º da Portaria RTR/UFGD n. 200, de 16/03/2020 e Art. 2º e 5º da Instrução Normativa PROPP/UFGD Nº 1, de 17/03/2020

1 Participação remota dos membros da banca conforme § 3º do Art. 1º da Portaria RTR/UFGD n. 200, de 16/03/2020 e Art. 2º e 5º da Instrução Normativa PROPP/UFGD Nº 1, de 17/03/2020

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APROVADA, conforme declarações anexas. Nada mais havendo a tratar, lavrou-se a presente ata, que vai assinada peo presidente da Comissão Examinadora.

Dourados, 29 de outubro de 2021.

Dr.ª Kely de Picoli Souza - PPGCS/UFGD _____
Dr. Paulo Nuno Centeio Matafome/FMUC (participação remota)
Dr. Nelson Luis de Campos Domingues/UFGD (participação remota)
Dr. Caio Fernando Ramalho de Oliveira/UFMS (participação remota)
Dr. Edson Lucas dos Santos - PPGCS/UFGD (participação remota)
Dr.ª Ana Margarida Coelho Abrantes/FMUC (participação remota)
Dr. Flávio Nelson Fernandes Reis/FMUC (participação remota)

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Pró-Reitoria de Ensino de Pós-Graduação e Pesquisa
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DEDICATION

To my mom Heloisa. Once again and forever for you, mom!

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EPIGRAPH

“Fall in love with studying,
this will make your dreams come true...”
(ULYSSES DRAGO DE CAMPOS)

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

$\cdot O_2^-$	Superoxide
$^1O_2$	Singlet oxygen
3T3-L1	Mouse embryonic fibroblast cell line
4HR	4-hexylresorcinol
8-OHdG	8-hydroxy-2-deoxyguanosine
AA	Ascorbic acid
ACh	Acetylcholine
ADP	Adenosine diphosphate
AGE	Advanced glycation products
AKT	Protein kinase B – same as PKB
AMPK	Adenosine monophosphate activated protein kinase
ARE	Antioxidant response elements
ATP	Adenosine triphosphate
AUC	Area under the curve
AV	Annexin V
BCA	Bicinchoninic acid
BHA	Butyl-hydroxy-anisole
BHT	Butyl-hydroxy-toluene
BSA	Bovine serum albumin
$CaCl_2$	Calcium chloride
CAT	Catalase
$CCl^{\cdot}_3$	Triclhoromethanide
CEBP α	CCAAT enhancer-binding protein α
CoQ	Ubiquinone
Cu	Copper
DAG	Diacylglycerol
DAPI	4',6-diamidino-2-phenylindoledihydrochloride
DCF	Dichlorofluorescein
DHE	Dihydroethidium
DM	Diabetes mellitus
DM1	Type 1 diabetes mellitus

DM2	Type 2 diabetes mellitus
DMEM-HG	Dulbecco's modified Eagle medium – high glucose
DNA	Deoxyrybonucleic acid
Dox	Doxorubicin
DPP4	Dipeptidyl peptidase-4
EA-Aa	Aqueous extract of *Acrocomia aculeata* leaves
EAT	Epididymal adipose tissue
ECL	Enhanced hemiluminescence
EDTA	Ethylenediamine tetraacetic acid
EGMTM-2	Endothelial Cell Growth Medium-2 Bullet Kit TM
EGTA	Ethylene-bis(oxyethylenenitrilo) tetraacetic acid
eNOS	Endothelial nitric oxide synthase
EpRE	Electrophile responsive element
ERK	Extracellular signal-regulated kinase
F-6-P	Fructose-6-phosphate
$FADH_2$	Flavin-adenine dinucleotide
FBS	Fetal bovine serum
FFA	Free fatty acids
G-6-P	Glucose-6-phosphate
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GFAT	Glutamine-fructose-6-phosphate-amidotransferase
GIP	Glucose-dependent insulinotropic peptide
GK	Goto-kakizaki rats
GK-Ctrl	Goto-kakizaki rats - control group
GK-EA-Aa	Goto-kakizaki rats - treated with aqueous extract of *Acrocomia aculeata* leaves
GlcN-6-P	Glutamine into glucosamine-6-phosphate
GLO-1	Glyoxalase-1
GLP-1	Glucagon-like peptide-1
GLUT	Glucose transporter
GPx	Glutathione peroxidase
GRd	Glutathione reductase
GSH	Reduced glutathione

GSSG	Oxidized glutathione
GST	Gluthatione S-transferase
H^+	Proton
H_2DCFDA	2',7'-dichlorodihydrofluorescein diacetate
H_2O	Water
H_2O_2	Hydrogen peroxide
H9c2	Rat cardiomyoblast cell line
HClO	Hypochlorous acid
HIF-1α	Hypoxia-inducible factor-1 alpha
HMOX1	Heme oxygenase-1
HMVec-D	Human dermal microvascular endothelial cells
4-HNE	4-hydroxy-2-nonenal
HNO_2	Nitrous acid
$\cdot HO_2$	Hydroperoxyl
IDPP4	Dipeptidyl peptidase-4 inhibitors
INRF2	Nuclear factor erythroid 2–related factor 2 inhibitor
IONOX-100	Di-tertbutyl-4- hydroxymethylphenol
IR	Insulin receptor
IRS	Insulin receptor substrate
ISGLT2	Sodium glucose co-transporter-2 inhibitors
ITT	Insulin tolerance test
JC-1	5,5,6,6-tetrachloro-1,1,3,3-tetraethyl benzimidazolocar-bocyanine iodide
JNK	c-Jun N-terminal kinases
K562	Human erythroleukemic cell line
KCl	Potassium chloride
Keap 1	Kelch-like ECH-associated protein 1
KH_2PO_4	Monopotassium phosphate
LPL	Lipoprotein lipase
MAPK	Mitogen-activated protein kinase
MCF-7	Human breast cancer cell line
MDA	Malondialdehyde
MEK	Mitogen-activated protein kinase kinase

METC	Mitochondrial electron transport chain
MG	Methylglyoxal
$MgSO_4$	Magnesium sulphate
ML385	Chemical NRF2 inhibitor
Mn	Manganese
mTOR	Mammalian target of rapamycin
N_2O_3	Nitrous oxide
N_2O_4	Dinitrogen tetraoxide
NA	Noradrenaline
Na_3VO_4	Sodium orthovanadate
NaCl	Sodium chloride
NAD	Nicotinamide adenine dinucleotide
NADH	Reduced nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NaF	Sodium fluoride
$NaHCO_3$	Sodium bicarbonate
NDGA	Nordihydroguaiaretic acid
NF-κB	Nuclear factor-κB
$NO^{\cdot}$	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NQO1	Quinone oxide reductase
NRF1	Nuclear respiratory factor 1
NRF2	Nuclear factor erythroid 2–related factor 2
O_2	Oxygen
O_3	Ozone
OG	Octyl gallate
$OH^{\cdot}$	Hidroxyl
$ONOO^-$	Peroxynitrite
PARP	Poly (ADP-ribose) polymerase
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PDX-1	Pancreatic duodenal homeobox-1

PG	Propil gallate
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator-1 alpha
PI	Propidium iodide
PI3K	Phosphatidylinositol-3-kinase
PKB	Protein kinase B
PKC	Protein kinase C
PMSF	Phenylmethylsulfonyl fluoride
PPARγ	Peroxisome proliferator-activated receptor gamma
PVDF	Polivinilideno
$Q^{\cdot -}$	Ubsemiquinone
QH_2	Ubiquinol
RAGE	Advanced glycation end product receptor
RBC	Red blood cells
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
$RO^{\cdot}$	Alkoxyl
$ROO^{\cdot}$	Peroxyl
ROOH	Organic hydroperoxide
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute Medium
RS	Reactive species
SDS	Sodium lauryl sulfate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SGLT2	Sodium glucose co-transporter-2
SIRT	Sirtuin
SOD	Superoxide dismutase
SUs	Sulfonylureas
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TBHQ	Terc-butil-hidroquinone
TBS	Tris buffer solution
TGF	Transforming growth fator
THBP	2,4,5-Trihydroxybutyrophenone

TOPO2β	Topoisomerase 2β
TZD	Thiazolidinedione
UDP-GlcNAc	Uridine 5'-diphospho-N-acetyl-D-glucosamine
VEGF	Vascular endothelial growth factor
W-Ctrl	Wistar rats – control group
W-EA-Aa	Wistar rats treated with aqueous extract of *Acrocomia aculeata* leaves
Zn	Zinc

Avaliação do potencial farmacológico do extrato aquoso das folhas de *Acrocomia aculeata* (Jacq.) Lodd ex. Mart.

RESUMO

O aumento da expectativa de vida da população mundial está diretamente relacionado à incidência de doenças crônicas como doenças neurodegenerativas, cardiovasculares, artrite reumatoide, diabetes, câncer, dentre outras. O impacto do tratamento destas doenças e a manutenção da saúde representam alto custo não só econômico, mas também social. O estresse oxidativo é um fator chave associado à etiologia e progressão dessas doenças. Assim, o desenvolvimento de alternativas terapêuticas é de fundamental importância, principalmente com baixo custo, fácil acesso e, acima de tudo, com relevante potencial farmacológico. As plantas medicinais, em especial as com potencial antioxidante, são uma importante fonte de compostos naturais que podem ser utilizadas como matéria-prima no desenvolvimento de novas terapias. A *Acrocomia aculeata* é uma palmeira nativa do Brasil, cujas folhas são ricas em compostos fenólicos e flavonoides, com comprovado efeito antioxidante. Desta forma, nosso objetivo foi avaliar o efeito do extrato aquoso das folhas de *A. aculeata* (EA-Aa) em duas condições relacionadas ao estresse oxidativo, as complicações associadas a diabetes e a cardiotoxicidade induzida pela quimioterapia com doxorrubicina (Dox). Para a investigação dos efeitos associados às complicações da diabetes, foram utilizados ratos Wistar normoglicêmicos e ratos Goto Kakizaki (GK), diabéticos tipo 2 não obesos, tratados diariamente com 200 $mg.kg^{-1}$ EA-Aa por 30 dias e avaliados em relação aos parâmetros antropométricos e bioquímicos. Após o tratamento, os animais foram anestesiados e eutanasiados. Proteínas associadas à hiperglicemia e ao estresse oxidativo foram avaliadas em órgãos-alvo em pré-adipócitos 3T3-L1, e em células microvasculares da derme (HMVec-D) induzidas ao desequilíbrio redox por H_2O_2. Os resultados mostraram que EA-Aa reduziu a glicemia em jejum e triglicerídeos de ratos diabéticos, através da melhora nos níveis de proteínas como GLUT-4, PPARγ e AMPK e reduziu o estresse oxidativo em pré-adipócitos 3T3-L1 e em células HMVec-D, além da promoção de níveis mais elevados de proteínas relacionadas à resistência ao estresse, SIRT1 e NRF2. Quanto aos efeitos de EA-Aa em associação com a Dox, um agente quimioterápico conhecido por sua alta cardiotoxicidade provocada pelo estresse oxidativo, foram utilizados modelos experimentais *in vitro*: células normais (células do sangue periférico PBMC e cardiomioblastos H9c2) e tumorais

(eritroleucêmicas K562 e câncer de mama MCF-7), e *in vivo*, camundongos C57Bl/6, tratados com EA-Aa, com ou sem Dox. O EA-Aa não apresentou toxicidade em células normais *in vitro* e no teste de toxicidade oral aguda *in vivo*. Em relação ao efeito de EA-Aa associado com a Dox, houve redução do estresse oxidativo induzido em eritrócitos e em células H9c2 e da cardiotoxicidade em camundongos C57Bl/6, além de potencializar o efeito citotóxico de Dox em células tumorais. Em conjunto, nossos resultados mostram a proteção antioxidante do EA-Aa nas complicações diabéticas e decorrentes do tratamento com Dox em vários tecidos, bem como seus mecanismos de ação. Assim, os dados aqui apresentados suportam novos estudos baseados no potencial farmacológico de EA-Aa e de seus fitoquímicos para o desenvolvimento de estratégias terapêuticas para o tratamento de ambas as condições.

Palavras-chave: estresse oxidativo, macaúba, bocaiúva, diabetes, câncer.

Evaluation of the Pharmacological Potential of the Aqueous Extract of the Leaves of *Acrocomia aculeata* (Jacq.) Lodd ex. Mart.

ABSTRACT

The increase in life expectancy of the world population is directly related to the incidence of chronic diseases such as neurodegenerative and cardiovascular diseases, rheumatoid arthritis, diabetes, cancer, among others. The impact of the treatment of these diseases and the maintenance of health represents a high economic and social costs. Oxidative stress is a key factor associated with the etiology and progression of these diseases. Thus, the development of therapeutic alternatives is of fundamental importance, especially with low cost, easy access, and, above all, with relevant pharmacological potential. Medicinal plants, especially those with antioxidant potential, are an important source of natural compounds that can be used as raw materials in the development of new therapies. *Acrocomia aculeata* is a palm native of Brazil, whose leaves are rich in phenolic and flavonoid compounds, with a proven antioxidant effects. Thus, our objective was to evaluate the effect of the aqueous extract of *A. aculeata* leaves (EA-Aa) in two conditions related to oxidative stress, the complications associated with diabetes and the cardiotoxicity induced by chemotherapy with doxorubicin (Dox). For the investigation of the effects associated with diabetes complications, normoglycemic Wistar rats and non-obese type 2 diabetic rats, Goto-Kakizaki (GK), were treated daily with 200 $mg.kg^{-1}$ EA-Aa for 30 days and evaluated in relation to the anthropometric and biochemical parameters. After treatment, the animals were anesthetized and euthanized. Proteins associated with hyperglycemia and oxidative stress were evaluated in target organs, in 3T3-L1 pre-adipocytes, and in dermal microvascular cells (HMVec-D) induced by H_2O_2 to redox imbalance. The results showed that EA-Aa reduced fasting glycemia and triglycerides of diabetic rats, by increasing the levels of proteins such as GLUT-4, PPARγ and AMPK and reduced oxidative stress in 3T3-L1 pre-adipocytes and HMVec-D cells, in addition to promoting higher levels of proteins related to stress resistance, SIRT1 and NRF2. Regarding the effects of EA-Aa in association with Dox, a chemotherapeutic agent known for its high cardiotoxicity caused by oxidative stress, *in vitro* experimental models were used: normal cells (PBMC peripheral blood cells and H9c2 cardiomyoblasts) and tumor cells (K562 erythroleukemics) and breast cancer MCF-7), and *in vivo*, C57Bl/6 mice, treated with EA-Aa, with or without Dox. EA-Aa showed no toxicity in normal cells *in vitro* and in the acute oral toxicity test *in vivo*. Regarding the effect of EA-Aa

associated with Dox, there was a reduction in oxidative stress induced in erythrocytes, in H9c2 cells and in cardiotoxicity in C57Bl 6 mice, in addition to potentiating the cytotoxic effect of Dox in tumor cells. Together, our results show the antioxidant protection of EA-Aa in diabetic complications and in those resulting from treatment with Dox in various tissues, as well as its mechanisms of action. Thus, the data presented here support new studies based on the pharmacological potential of EA-Aa and its phytochemicals for the development of therapeutic strategies for the treatment of both conditions.

Keywords: oxidative stress, *macaúba, bocaiúva*, diabetes, cancer.

SUMMARY

1 INTRODUCTION

The increase in life expectancy of the population is directly related to a higher incidence of chronic diseases, with a considerable negative impact regarding the comorbidities and medical costs (MONTEIRO-ALFREDO et al., 2020a; VATNER et al., 2020). Oxidative stress, characterized by an imbalance in the body's redox balance, is a key factor related to the etiology and progression of diseases, being related to physiological and genetic factors and the new habits adopted by the population (PISOSCHI et al., 2021). Under physiological conditions, oxidative stress, known as 'oxidative eustress', helps the body to function in redox signaling, acting on specific targets. In contrast, in supra physiological conditions, it leads to the impairment of redox signaling and the consequent damage to the organism's biomolecules, namely, carbohydrates, lipids, proteins and nucleic acids, culminating in pathophysiological alterations (SIES, 2019), such as cardiovascular and neurodegenerative diseases, rheumatoid arthritis, cancer and type 2 diabetes mellitus (DM2) (PISOSCHI et al., 2021).

The development of therapeutic alternatives is of crucial importance, especially if they have features that include low cost, easy access, low toxicity, and above all, a relevant pharmacological potential (MONTEIRO-ALFREDO et al., 2020a). According to the World Health Organization (WHO), medicinal plant consumption in developing countries is around 65% to 80% for primary health care (WHO, 2011). More than 50% of the new drugs currently developed are derived from medicinal plants or its constituents (TENG; SHEN, 2015). Even with this considerable percentage, they represent only 15% of the 300,000 species of existing plants in the world (DE LUCA et al., 2012). Thus, medicinal plants are a great source of potential chemical compounds available for the development of new alternatives (DE CARVALHO et al., 2020).

Brazil possesses the greatest biodiversity worldwide, with a vast fauna and flora and important biomes, such as Cerrado (FONSECA; VENTICINQUE, 2018), which makes it a meaningful target regarding the research of new alternatives to produce plant-based products, such as foods, cosmetics and medicines (CARVALHO; CONTE-JUNIOR, 2021). Regarding the therapeutic potential of Cerrado plants, this has already been described for several species that associate ethnopharmacological knowledge with relevant and promising phytochemical compounds and therapeutic effects. *Acrocomia aculeata* Jacq. (Lodd) ex Mart is a palm native from Brazilian Cerrado, popularly known as *macaúba* or *bocaiúva* (CÉSAR et al., 2015), which has economic importance due to its versatility, its use in food (COSTA; OLIVEIRA; COSTA,

2018), biodiesel (CÉSAR et al., 2015; SOUZA et al., 2016; TEIXEIRA et al., 2017), and cosmetics production (DARIO et al., 2018; DE SOUZA et al., 2020; DEL RÍO et al., 2016). In addition, several studies reported the pharmacological potential of its pulp in the treatment of respiratory diseases, besides its analgesic and laxative effects (AGOSTINI-COSTA, 2018), and the potential in reducing serum glucose and cholesterol levels (SILVA, 2012).

Recently, our group demonstrated the antioxidant potential of *A. aculeata* leaves, which has a strong antioxidant effect, mainly based on its phenolic compounds, vanillic, gallic, ferulic and caffeic acids, and the flavonoids rutin, and quercetin (MONTEIRO-ALFREDO et al., 2020a). In this perspective, our goal was to evaluate the effect of the aqueous extract of *A. aculeata* leaves (EA-Aa) in two oxidative stress-related conditions: 1) complications associated to DM2 and; 2) cardiotoxicity induced by chemotherapy performed with doxorubicin (Dox). According to this, we aimed to assess whether the antioxidant potential of *A. aculeata* leaves might improve the metabolic profile of diabetic rats, as well as to unravel the underlying mechanisms in tissues involved in glucose metabolism and affected by hyperglycemia-associated complications. We also aimed to evaluate the association between EA-Aa and Dox chemotherapy in *in vitro* and *in vivo* experimental models, to evaluate its potential as a pharmacological adjuvant, to improve Dox efficacy reducing the side effects linked to oxidative stress, to promote a better quality of life for patients and a greater adherence to treatment.

2 BACKGROUND

The redox imbalance caused by oxidative stress promotes damage in biomolecules, tissues, and organs. Such modification acts as a trigger for altering the functioning of the organism, leading to the onset of diseases, and altering people's quality of life (MONTEIRO-ALFREDO et al., 2020a; VATNER et al., 2020). Among the various diseases that have a direct relation with oxidative stress, we highlight DM2 and cancer. Both DM2 and cancer are non-communicable diseases, which constitute 7 in each 10 causes of death in the world, according to the World Health Organization (WHO, 2020). DM2 is the most common type of diabetes, and in recent decades its prevalence has dramatically increased, especially in developing countries, affecting 422 million of people, with 1.6 million deaths per year (WHO, 2021a). Cancer is the second global cause of death, with an estimative of 10 million deaths in 2020 (WHO, 2021b).

DM2 is related to oxidative stress through pathways associated with hyperglycemia toxicity, what leads to its complications (BROWNLEE, 2005). The anticancer therapies make use of several drugs with a remarkable increase in oxidative stress as a side effect, such as doxorubicin (Dox), a drug widely used in the treatment of various types of cancer. The increase in ROS caused by the cumulative administration of Dox leads to chronic cardiomyopathy and cardiac failure (CAPPETTA et al., 2017). In this scenario, the development of new alternatives of treatment is essential, considering the high adherence of the population to plant-based treatments. The fact that Brazil is a country with great biodiversity, and the great diversity in the chemical composition of plants, may lead to the identification of significant pharmacological effects. Therefore, research with Brazilian plants is an important strategy that can help in identifying therapeutic strategies able to maintain health and treat oxidative stress-related diseases.

2.1 Oxidative stress

The first theory related to oxidative stress was enunciated in the early 1950s. It was proposed by a biochemist, Denham Harman, from University of Nebraska, but only in the late 1960s that it was started to be accepted (MEHDI; SOLANKI; SINGH, 2021)**.** The definition consisted in the continuous and inevitable attack and consequent damage of cells by free radicals which spread through tissues and organs and could even establish a degenerative

disease. Harman stated that this condition was influenced by genetic and environmental factors and that it was part of the aging process (HARMAN, 2006). In the 1980s, Professor Helmut Sies, considered as the redox pioneer (MAJIMA et al., 2016), updated the definition of this condition to 'oxidative stress', and described the imbalance between cellular defense mechanisms, and the triggering oxidative reactions, that is the imbalance between the body's pro-oxidant and the antioxidant system (CADENAS; SIES, 1985). Later, with the advancement of studies in the redox area, this concept was improved and approached to the real definition, and currently it is defined as the increase in reactive species (RS), in parallel with the reduction of antioxidant defense (SUBRAMANIAM et al., 2020).

RS are divided in two groups, radical (free radicals) and nonradical, and the most common are formed from oxygen and nitrogen. The reactive oxygen species (ROS) can be represented by singlet oxygen ($^1O_2$), hydroxyl ($OH\cdot$), hydroperoxyl ($\cdot HO_2$), superoxide ($\cdot O_2^-$), organic hydroperoxide (ROOH), alkoxyl ($RO\cdot$) and peroxyl ($ROO\cdot$) radicals; the non-reactive oxygen are hypochlorous acid (HClO) and hydrogen peroxide (H_2O_2). The reactive nitrogen species (RNS) are dinitrogen trioxide (N_2O_3), dinitrogen tetraoxide (N_2O_4), nitrous acid (HNO_2), nitric oxide ($NO\cdot$), nitrites (NO_2^-), nitrates (NO_3^-), and peroxynitrite ($ONOO^-$) (PHANIENDRA; JESTADI; PERIYASAMY, 2015).

There are also sulfur (HALLIWELL; GUTTERIDGE, 2015), chlorine, carbon, and transition metals-derived, which also have biological importance in the context of RS (FINKEL; HOLBROOK, 2000). During the process of electronic stabilization, as detailed above, RS attack other molecules through several mechanisms (NEHA et al., 2019), acting in biochemical, physiological, and pathological processes of the body such as cell signaling, neurotransmission (YAMAMOTO; GAYNOR, 2001), and immune defenses (ZHANG et al., 2013). In general, they assist in homeostasis when they are in balanced amounts of production and neutralization. However, the imbalance between them generates a pro-oxidant state and, as a consequence, oxidative stress (VASCONCELOS et al., 2007). RS are also essential for various functions of the organism and the excessive generation of RS is neutralized by the body's antioxidant defense system to maintain healthy conditions (BONILHA, 2018). In this review, we mainly will discuss ROS.

2.1.1 Electron transport chain and the formation of ROS

The mitochondrial electron transport chain (METC, Figure 1) is one of the main ROS generating centers. The mitochondria are crucial organelles for cellular homeostasis, acting in the production of adenosine triphosphate (ATP) and, consequently, in ROS formation. ROS are mostly generated during the several electron transfers that occur between their complexes (I, II, and III, as mentioned below) (NOLFI-DONEGAN; BRAGANZA; SHIVA, 2020). During the transport of electrons between mitochondrial complexes, an "escape" occurs and consequently ROS are formed, constituting the primary source of $\cdot O_2^-$ in most tissues (TURRENS, 2003). They may be dismuted into H_2O_2, and later into other ROS (NOLFI-DONEGAN; BRAGANZA; SHIVA, 2020) as we describe later. Physiologically, ROS are formed to support in various immunological and signaling processes in the organism, but they can also be involved in pathological processes, when found in excess (MONTEIRO-ALFREDO et al., 2020a; TURRENS, 2003).

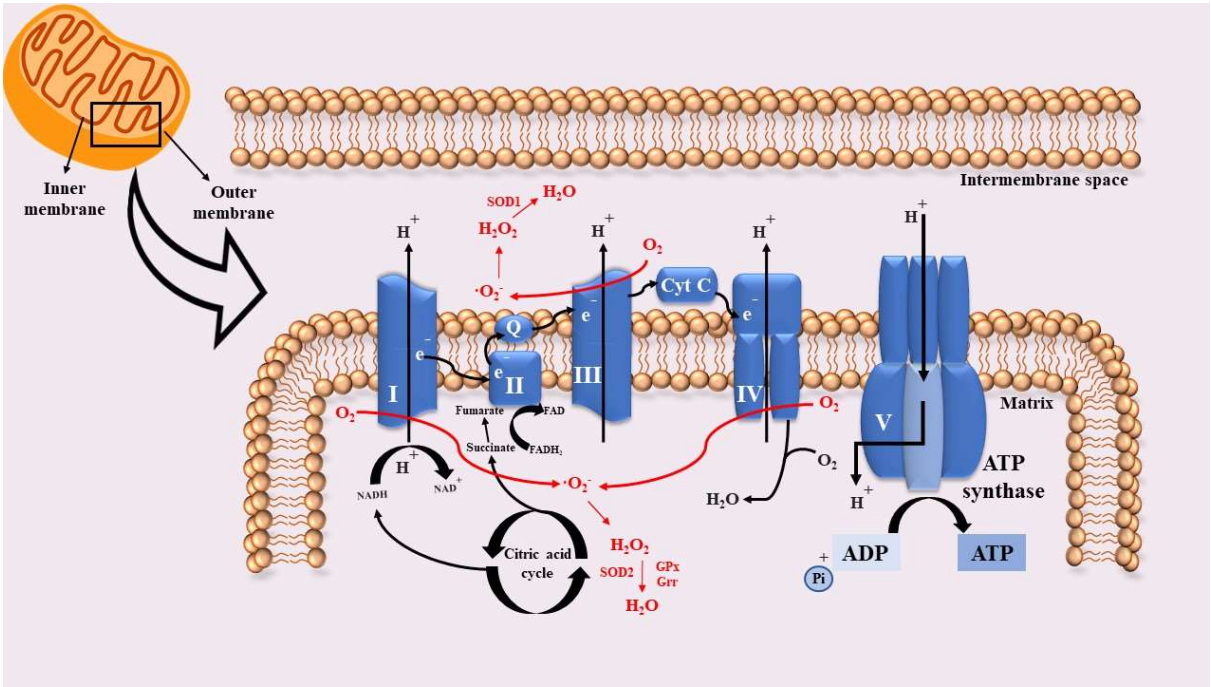


Figure 1: Mitochondrial electron transport chain (MONTEIRO-ALFREDO, 2021).

The energy supply (ATP) needs of each tissue varies. Endothelial cells, for example, are more dependent on glycolysis as an energy source than cardiomyocytes, as they need more than 95% of ATP provided by mitochondria for their functioning. However, regardless of whether the energy requirement comes from different sources, the production of ROS and ATP are linked by METC (NOLFI-DONEGAN; BRAGANZA; SHIVA, 2020). Thus, the intracellular

levels of glucose are a key regulator of METC activity and, thus, ROS generation (BROWNLEE, 2005).

The electron transport chain is found in the inner membrane of the mitochondria, close to the mitochondrial matrix where the Krebs cycle is located. The Krebs cycle provides nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) for METC, respectively, for complex I (NADH: ubiquinone oxidoreductase) and complex II (succinate dehydrogenase). In the sequence, electrons from NADH are transferred to coenzyme Q (CoQ) (ubiquinone) with the help of several cofactors in complex I to enter in the Q cycle, which reduces CoQ to ubiquinol (QH_2 – CoQ reduced form). These two-electron transfer process promote the pumping of four protons (H^+) from complex I to the intermembrane space. Complex II can also serve as a gateway for electrons; in this case, they are donated by $FADH_2$ and transferred to CoQ, but it is not accompanied by H^+ translocation as in complex I (NOLFI-DONEGAN; BRAGANZA; SHIVA, 2020).

In the Q cycle the electrons go to complex III (coenzyme Q: cytochrome c reductase) and later to cytochrome c. For this, QH_2 binds to complex III on its cytoplasmic side and releases two H^+ in the intermembrane space. One electron goes from QH_2 to an iron-sulfur cluster of complex III, while the second electron goes to cytochrome b within complex III, which goes to another Q molecule bounded to the matrix of the complex to form a ubsemiquinone ($Q \cdot^-$). At the same time, the electron present in the iron-sulfur cluster move to the cytochrome, and another QH_2 molecule binds to the membrane side of the complex and undergoes an oxidation process, reducing $Q \cdot^-$ to QH_2 again, thus completing the Q cycle, and pumping another pair of H^+ into the intermembrane space of the mitochondria (NOLFI-DONEGAN; BRAGANZA; SHIVA, 2020).

As soon as cytochrome c is reduced, it starts to transport single electrons from complexes III to IV (cytochrome c oxidase), which bind to molecular oxygen and are reduced to water. Complex IV, on the other hand, consists of three subunits and is where eight H^+ are pumped from the matrix, four of them are used to generate two water molecules and four go to the intermembrane space. This oxygen consumption process describes the mitochondrial respiration. At the end, the H^+ are transported through METC, and the electrons from complex I to IV. They are pumped from the matrix to the intermembrane space, where they accumulate to form an electrochemical gradient of H^+, known as the mitochondrial membrane potential ($\Delta\Psi$) (NOLFI-DONEGAN; BRAGANZA; SHIVA, 2020).

ΔΨ associated with H^+, which generates a protonmotive force Δp, is essential for energy storage during mitochondrial oxidative phosphorylation. It associates all electrons transported by complexes I to IV and the oxygen expenditure in complex V (ATP synthase), which are pumped from the matrix of the mitochondria to the intermembrane space because of the H^+ gradient generated. Complex V is composed by subunits of distinct extra and intramembranous domains, which act as a rotational engine that allows the production of ATP, this rotation is promoted by the H^+ gradient generated. The mechanism of ATP formation occurs with the rotation of the complex V, resulting in the addition of phosphate to adenosine diphosphate (ADP), thus forming ATP (AHMAD; WOLBERG; KAHWAJI, 2021; NOLFI-DONEGAN; BRAGANZA; SHIVA, 2020; TURRENS, 2003).

In the entire METC process, the generation of ROS occurs mainly in complexes I and III, by reverse electron transport in complex I, and the reduction based on the iron-sulfur cluster in complex III. Complex II, despite having a less representative ROS production rate, is also responsible for mitochondrial ROS production (NOLFI-DONEGAN; BRAGANZA; SHIVA, 2020). An additional fact that must be considered for the generation of ROS is the availability of oxygen to receive electrons, although there are studies that demonstrate the production of ROS in hypoxia (WAYPA; SCHUMACKER, 2008).

2.1.2 ROS and the oxidative context

Oxidative events are associated with physiological (normal aging) and pathological conditions, and the cascade of events that regulate and determine the conditions that will be associated with these processes are based on the formation of ROS (RADI, 2018). Under physiological conditions, a small portion of the oxygen (2 to 5%) undergoes a univalent reduction, where it receives only one electron, located in the external orbitals, making it unpaired and highly reactive (HALLIWELL; GUTTERIDGE, 1989). Most ROS produced by aerobic organisms are generated in membrane nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), and in the METC (ZHANG; WANG; CHU, 2019), as shown in Figure 1. However, there are some other sources; the endogenous are xanthine oxidase, phagocytosis, arachidonate pathways, physical exercise (LOBO et al., 2010), inflammatory process, cyclooxygenases, lipoxygenases, cytochrome p450 enzymes (FINKEL, 2011), and the β-oxidation of external lipid segments in peroxisomes (ERSHOV; BAZAN, 2000). The exogenous are the exposure to radiation, environmental pollutants, some drugs and solvents

(LOBO et al., 2010), and the ‘new habits’ of the population that include smoking, high fat diet (HFD), among others (CANO et al., 2010). HFD is directly associated to higher amounts of fatty acids intake, causing an imbalance in metabolism (TRINDADE DE PAULA et al., 2016) and the activation of proinflammatory pathways related to adipose tissue expansion, what culminates with the increase in lipoperoxidation biomarkers linked to oxidative stress and deficiencies that can lead to the development of metabolic syndrome (TAN; NORHAIZAN, 2019).

Molecular oxygen in the ground state contains two unpaired electrons on the outside, the known triplet oxygen (TURRENS, 2003). When oxygens have the same spin, they have the ability to react only one at a time, being characterized as low reactivity (regarding chemical reactions). Reactions where one of the two electrons is excited and changes its rotation generate singlet oxygen, a highly oxidizing agent that possesses two electrons rotating in opposite directions and react quickly with other pair of electrons, especially in double bonds (TURRENS, 2003). In older cells, the generation of ROS is higher in comparison to the newer ones, and in cases of mutations, these levels are even higher (JABBEHDARI; HANDA, 2021). The production can increase up to 10 fold in damaged mitochondria (GRIVENNIKOVA; KAREYEVA; VINOGRADOV, 2010).

2.1.3 Antioxidant detoxification system

The body’s antioxidant system exists to detoxify excessive ROS to prevent the development of oxidative stress and its consequences. It is composed by several enzymes that act to neutralize the ROS formed (LOBO et al., 2010). Antioxidants are compounds that, when in low concentrations in relation to the oxidizing agent, neutralize the substrate or act in preventing its formation, avoiding subsequent oxidation (HALLIWELL; GUTTERIDGE, 2015). There is a vast number of antioxidants, both endogenous and exogenous, available in the environment and on the market. Endogenous antioxidants can be classified as enzymatic and non-enzymatic. The enzymatic system is formed by the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) (BARBOSA et al., 2010), whereas the non-enzymatic system is represented by uric acid, lipoic acid, melatonin, bilirubin, and glutathione (GSH).

Regarding exogenous antioxidants, they can also be divided into two groups, natural and synthetic. Natural ones are represented by phenolic compounds, carotenoids, flavonoids,

vitamins E, A, and C, for example (NEHA et al., 2019), and they neutralize ROS from metabolism and exogenous sources, which consequently impair the damage of macromolecules. In comparison with natural antioxidants, the synthetic antioxidants are more effective, stable, and cheaper than the naturals, but their uses are associated with different side effects (FASSEAS et al., 2008). They have been used as additives to prevent oxidation in biodiesel (JEMIMA ROMOLA et al., 2021), cosmetics, foods, lubricants, and in pharmaceutical industry (HAM et al., 2019), and the main representatives in this class are butylhydroxy-anisol (BHA), butylhydroxy-toluene (BHT) (RAMALHO; JORGE, 2006), tertiary butyl hydroquinone (TBHQ), 2,4,5-trihydroxybutyrophenone (THBP), propyl gallate (PG), octyl gallate (OG), di-tertbutyl-4- hydroxymethylphenol (IONOX-100), 4-hexylresorcinol (4HR), and nordihydroguaiaretic acid (NDGA) (XIU-QIN et al., 2009). A further protection provided by antioxidants besides the neutralization of ROS includes the repair of the damage caused by them, and the replacement of damaged biological structures, providing an adaptation of the organism itself, increasing the synthesis of antioxidant enzymes, and reducing the oxidative damages (BIANCHI; ANTUNES, 1999).

2.1.3.1 Enzymatic endogenous antioxidants

The detoxifying effect of antioxidants (Figure 2) occurs on RS, which are unstable intermediates generated by the reduction of oxygen by an electron (as the case of ROS). Initially, the $\cdot O_2^-$, formed by the reduction of oxygen, acts as a mediator of the METC, in addition to being the precursor to the formation of ROS. Its dismutation can occur spontaneously or through a reaction catalyzed by an enzyme that belongs to the group of SOD enzymes, leading to the formation of H_2O_2, which in turn can be totally reduced to water, or partially reduced to $OH^{\cdot}$, one of the most reactive existing ROS (TURRENS, 2003). The $OH^{\cdot}$ formation process is catalyzed by transition metals, such as iron and copper in the Fenton reaction (RADI, 2018) and in turn can form again the $\cdot O_2^-$ that can end up reacting with NO, forming $ONOO^-$ (RADI; CASSINA; HODARA, 2002) and other RNS (TURRENS, 2003), and the consequent biological responses, especially about the mitochondrial functioning (RADI; CASSINA; HODARA, 2002). The mitochondrial matrix contains a form of SOD composed with manganese on its active site, and it eliminates O_2 formed in both the matrix and the inner side of the mitochondria membrane (TURRENS, 2003). We explore in more detail the detoxifying mechanism and other enzymes involved in the process hereafter.

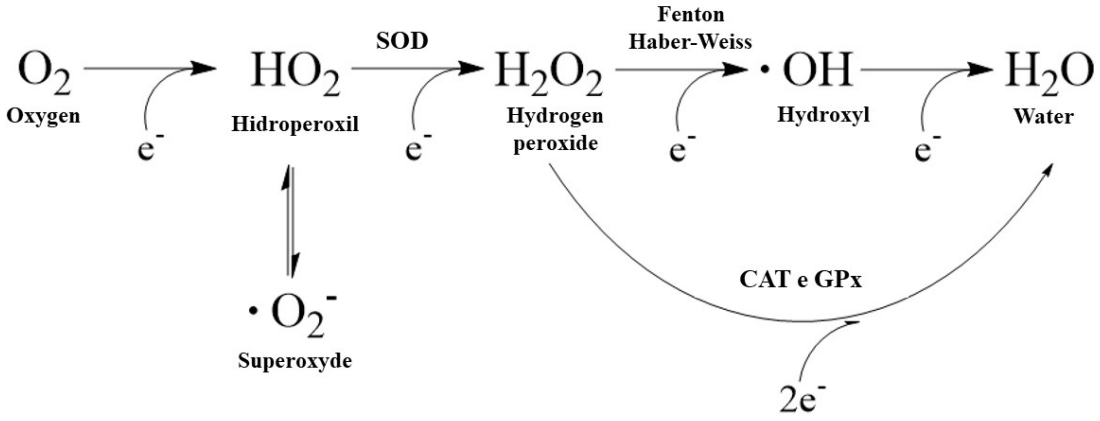


Figure 2. Mechanisms of action of antioxidant enzymes. During cellular respiration, the removal of some electrons causes the molecular conversion of oxygen (O_2) to the hydroperoxyl radical ($\cdot HO_2$), which is easily converted to the superoxide radical ($\cdot O_2^-$), which is highly reactive. This anion is neutralized by the action of superoxide dismutase (SOD), on hydrogen peroxide (H_2O_2). The hydrogen peroxide is converted to the water molecule with the aid of the Fenton reaction combined with two electrons. It can also be converted to water by the enzymes catalase (CAT) and glutathione peroxidase (GPx) (MAURYA et al., 2016).

It is important to emphasize the importance of the functioning of the entire detoxification system, as there are ROS that do not have a specific enzyme that acts in its neutralization, but rather need the joint action of the body's antioxidant mechanism. One example of this is $OH^{\cdot}$, a ROS that has no specific enzyme defense and has the highest reactivity, consequently it has a greater potential for oxidative damage (LEDO et al., 2009). $OH^{\cdot}$ is the main responsible for the initiation of the lipid peroxidation process, often changing the biological function of several macromolecules. Considering its characteristics and the absence of specialized defense, it is crucial a fine balance between antioxidant enzymes, so that the integral functions of cells are maintained and ROS neutralized (AYALA; MUÑOZ; ARGÜELLES, 2014).

2.1.3.1.1 Superoxide dismutase (SOD)

Among several functions, many studies have indicated that SOD has an important role in the prevention of oxidative stress during aging (RIZVI; MAURYA, 2007). It is the first enzyme that acts in the defense of cells against ROS, by catalyzing the dismutation process of $\cdot O_2^-$ into H_2O_2 (FAVARETTO et al., 2011). Its activity depends on enzymatic cofactors, which

differ according to the cell compartment in which they act. In the case of SOD, it can be found in several forms, SOD-Cu/Zn, associated and stabilized by copper and zinc, located in the cytoplasm and in all eukaryotic cells; SOD-Mn, which has manganese as a cofactor and is found in the mitochondria (BARBOSA et al., 2010) and peroxisomes; and SOD-Fe stabilized by iron, also found in peroxisomes (LOBO et al., 2010). The SOD isoforms present in the human body are SOD1, SOD2, and SOD3, located in the cytoplasm, mitochondrial, and extracellular medium, respectively. SOD1 and SOD3 have copper and zinc and SOD2 has manganese (LOBO et al., 2010).

2.1.3.1.2 Catalase (CAT)

CAT is divided into three groups based on their function and structure. They are typical catalases, catalase peroxidase and manganese catalase. The typical catalases are part of the group that is found in plants and animals (GALASSO et al., 2021). It is an important endogenous antioxidant enzyme that inhibit/neutralizes the formation and increase the removal of ROS/RNS (MAES et al., 2011). After the action of SOD, which catalyzes the exchange of $\cdot O_2^-$ into H_2O_2, a dangerous product originated by several metabolic processes, CAT and GPx act in sequence, reducing H_2O_2 to water and oxygen (LOBO et al., 2010; MAURYA et al., 2016). According to some authors, it is primarily responsible for the conversion of water and oxygen and it also does so when there is an oxidative imbalance (HALLIWELL; GUTTERIDGE, 1985). CAT is present in most aerobic cells and in the main human tissues, mostly found in peroxisomes (GALASSO et al., 2021). In animals, it is found in the liver (LOBO et al., 2010), kidneys and erythrocytes with high activity, while kidney, lung, pancreas and adipose tissue have an intermediate activity (GALASSO et al., 2021). Organs such as brain, heart and skeletal muscle also have CAT, although in small amounts (HALLIWELL; GUTTERIDGE, 1985).

2.1.3.1.3 Glutathione system

The glutathione system is composed by GSH, GSR, GPx and GST. It is present in microorganisms, animals, and plants (LOBO et al., 2010). GPx exists in different isoforms, selenium-dependent and nondependent, located in the cytoplasm or mitochondria (BARBOSA et al., 2010), and uses glutathione as a substrate to neutralize H_2O_2. This H_2O_2 can also be

converted into water molecules by the reaction of Fenton and Haber-Weiss (MAURYA et al., 2016), with the aid of iron and copper, which culminates in the generation of $OH^{\cdot}$ (BARBOSA et al., 2010) as schematically presented in Figure 3.

When H_2O_2 is present in physiological concentrations, GPx acts to transform it into water (HALLIWELL; GUTTERIDGE, 1985). Additionally, GPx depends on the redox cycle of glutathione, which involves reduced (GSH) and oxidized (GSSG) glutathione (BARBOSA et al., 2010), and the supply of hydrogen and electron cations carried out by NADH, FADH, and CoQ (HALLIWELL; GUTTERIDGE, 1989).

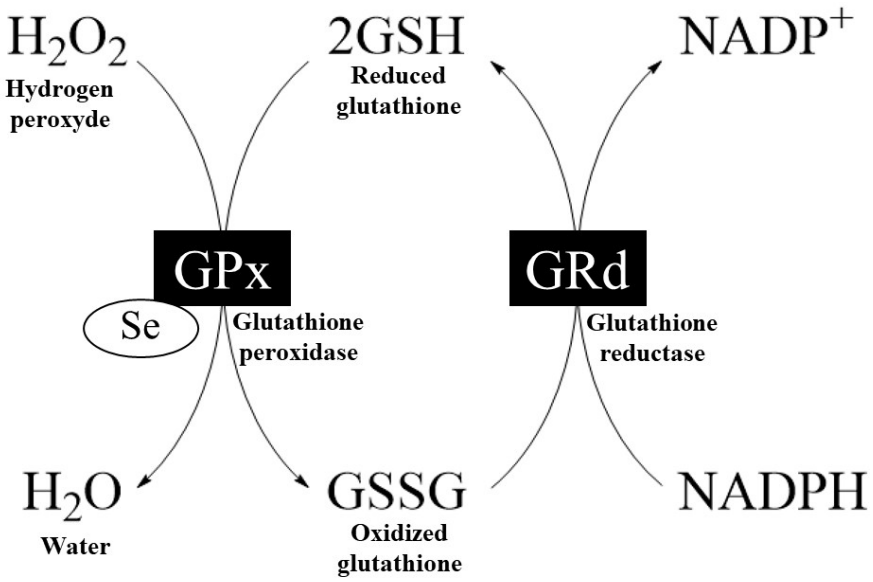


Figure 3. Redox glutathione cycle. For GPx reduces H_2O_2 to water, it needs GSH to be converted to GSSG, in sequence, GRd promotes the recovery of GSH, enabling the maintenance of the cycle and neutralization of reactive species (BARBOSA et al., 2010).

2.2 Oxidative stress and diabetes

Diabetes mellitus (DM) is a chronic metabolic disease characterized by destruction (type 1 – DM1) or dysfunction (type 2 – DM2) of pancreatic β-cells, resulting in hyperglycemia (JIMENEZ-LUNA et al., 2020). Here, our focus is DM2, which currently affects 463 million people and leads to an expenditure of 760 billion dollars in 2019 (corresponding to 10% of the expenses with health). In addition, 374 million people are at risk to developing DM2 and it is estimated that in 2045 more than 700 million people will be affected worldwide (‘International

Diabetes Federation - Facts and Figures', 2019). In addition, the prevalence of the disease may double in the next 20 years, considering the increase in age and especially the metabolic changes resulting from the bad lifestyle habits currently adopted by people, which culminate in metabolic changes and DM2 and its associated complications (MARÍN-PEÑALVER et al., 2016).

2.2.1 DM2 pathophysiology

DM2 is the most common metabolic disorder, usually caused by the lack of sensitivity of insulin-sensitive cells and loss of mass and function of the pancreatic β cells (GALICIA-GARCIA et al., 2020). Chronic insulin resistance becomes DM2 when β cells are unable to produce enough amounts of insulin to compensate the lower sensitivity of cells to its action (FU; GILBERT; LIU, 2013). Due insulin resistance (better explained below), there is an increase in hepatic glucose production and reduced glucose uptake in several organs (adipose tissue, liver, and muscle).

As the process related to insulin release and effect are crucial for the maintenance of glucose homeostasis, they are highly regulated and since there is a defect or change in this mechanism, a metabolic imbalance can set in, developing DM2. Risk factors triggering DM2 can include genetics, such as ethnicity and family history, and metabolic as well as environmental factors such as obesity, poor nutritional value of the diet and sedentary lifestyle (GALICIA-GARCIA et al., 2020).

2.2.2 Insulin synthesis and secretion

Insulin is a hormone produced in pancreatic β cells and acts as an important regulator of metabolism. Its synthesis occurs as pre-pro-insulin, which is processed as proinsulin, then converted to insulin and C-peptide, and later included in the secretary granules to be released. Insulin biosynthesis is stimulated by several factors, but glucose metabolism (explained below) is the most critical physiological factor that initiates the process of insulin gene transcription and mRNA translation (FU; GILBERT; LIU, 2013). Free fatty acids can also act to stimulate insulin production and it has recently been discovered that pancreatic β cells have a free fatty acid receptor, FFA2 (VILLA et al., 2016), through which they can act by stimulating the

production and release of the hormone. Amino acids can also influence this process, but less noticeable (FU; GILBERT; LIU, 2013).

Insulin secretion is a process that occurs from the fusion of insulin granules with the cell plasma membrane and subsequent exocytosis of the granular content. This process appears to occur in two phases, according to plasma glucose levels. In humans, the first phase of secretion starts when blood glucose reaches a concentration of ~126mg.dL^{-1}, with a release of 1.4 nmol.min^{-1}, and after ~10 minutes, the secretion decreases to 0.7 nmol.min^{-1} (FU; GILBERT; LIU, 2013).

The secretion process occurs following the oxidation of glucose by glucokinase enzyme, which increases ATP levels in the ratio ATP/ADP and NADH/NADPH and H^+ concentrations. Thus, ATP-sensitive K^+ channels close and inhibit K^+ efflux, depolarizing the β cell. The voltage-dependent Ca^{2+} channel opens, increasing the intracellular Ca^{2+} concentration, starting the exocytosis process of insulin within the granules, which move through microtubules, fusing the granules with the cell membrane, insulin undergoes exocytosis in the form of heterodimers, that dissociate into dimers, and monomers, the biological active ones (BARG; RORSMAN, 2004). When insulin is secreted, it remains freely circulating in the blood and has an average half-life of 6 minutes. Insulin molecules that do not bind to its receptor are degraded by insulinase, an enzyme that catalyzes the hydrolysis of insulin (MIRSKY; PERISUTTI, 1957)**,** currently called insulin-degrading enzyme (IDE), a metalloproteinase that is located mainly in the liver and kidneys. Insulin degradation also occurs in fibroblasts, adipocytes, gastrointestinal cells, lymphocytes, monocytes, and others. When bound to its receptor, it is internalized by endosomes along with IDE, which is responsible for its degradation (PIVOVAROVA et al., 2016).

2.2.3 Glucose metabolism

The increase in blood glucose resulting from the uptake of glucose and the consequent action of insulin in different organs is crucial for glycemic homeostasis in the body (KARYLOWSKI et al., 2004). Its regulation occurs according to the number of facilitating glucose transporters (GLUT) in the plasma membrane and its stimulation by insulin, which promotes glucose uptake from the blood through the subcellular translocation of GLUT until the cell membrane surface (defects in this mechanism are responsible for the installation of conditions such as DM2 and metabolic syndrome, as already mentioned) (BREWER et al.,

2014). There are 14 different types of GLUTs encoded by SLC2 gene, members of a superfamily of glucose membrane transport facilitators (MUECKLER; THORENS, 2013). They are located in different parts of the body, like GLUT2, present in hepatocytes, pancreatic islets, and retina (MEDINA; OWEN, 2002) and GLUT4, highly expressed in adipose tissue, muscle and cardiomyocytes (ALGHAMDI; ALSHUWEISHI; SALT, 2020). The isoforms of GLUT1, GLUT3, and GLUT5 are also studied, but they have a greater relation with the immune system and their regulation is made to meet the specific metabolic needs of each cell in the immune and inflammatory responses (FU et al., 2004). GLUT1 is also abundantly found in erythrocytes and brain, GLUT3 in brain, GLUT5 found in intestine, kidney, testis, and erythrocytes, GLUT6 in spleen, brain, and leukocytes GLUT7 in liver, GLUT8 in testis and brain, GLUT9 in kidney and liver, GLUT10 in liver and pancreas, GLUT11 found in heart and muscle and GLUT12 in heart and prostate (MEDINA; OWEN, 2002), and GLUT13 found in brain and adipose tissue and the last one, GLUT14 found in testis (MUECKLER; THORENS, 2013). The physiological justification for the existence of so many isoforms is due to the crucial importance of this sugar for human energy maintenance, which requires several glucose transporters with different kinetic properties, suitable for different cellular needs (MUECKLER; THORENS, 2013).

Insulin stimulation is paramount for GLUT translocation, and its production is initiated by the increase in glucose levels. Under basal conditions, GLUT4 levels are less than 5% in the cell membrane of primary adipocytes, for example, and when it increases, insulin has the role of increasing the GLUT4 translocation by up to 40-fold (BREWER et al., 2014). There are two cascades of proteins that are activated by insulin, but regarding glucose metabolism, those responsible for this process are part of the PI3K/AKT/mTOR signaling pathway, modulating glucose uptake, glycogen synthesis, differentiation, and cell growth. Its major metabolic effects are triggered by phosphorylation of its receptor (IR) and substrates of the receptor (IRS) (LETO; SALTIEL, 2012). On the other hand, the other activated pathway is the RAS/RAF/MEK/ERK, which has predominantly mitogenic effects, activated by IR phosphorylation of Shc (a type of IRS) and works together with the PI3K/AKT/mTOR pathway to control cell differentiation, growth, and apoptosis (ASANO et al., 2007; CHEUNG; MCDONNELL; HAMNVIK, 2021).

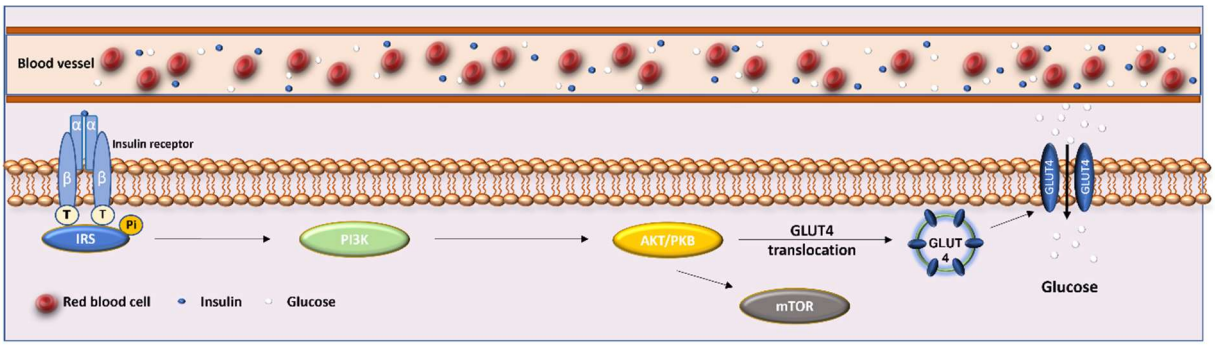


Figure 4. Insulin activation pathway (MONTEIRO-ALFREDO, 2021).

Thus, the first step of insulin action starts with the activation of IR, a transmembrane glycoprotein. This action occurs through its extracellular domain by the intrinsic activity of tyrosine kinase, which phosphorylates several IRS (ASANO et al., 2007). Phosphorylated IRS functions as a binding site for the PI3K enzyme, as it activates the protein kinase AKT or protein kinase B (PKB), which leads to activation of other targets until GLUT translocation to the cell surface (TEIXEIRA; FARIA, 2021). AKT is also related to the activation of the mammalian target of rapamycin (mTOR), as mentioned above. This target acts mainly on protein synthesis, cell proliferation, and inhibition of autophagy, and despite being fully involved in glycemic control, it is a positive regulator of pancreatic β cell function (RACHDI et al., 2008) and provides negative feedback for IRS, especially in mTOR1, while mTOR2 acts to inhibit gluconeogenesis (Figure 4) (CHEUNG; MCDONNELL; HAMNVIK, 2021; LAMMING et al., 2012). Glucose is mostly metabolized via the glycolytic pathways, which occurs through glycolysis, Krebs cycle, and the METC (FERNIE; CARRARI; SWEETLOVE, 2004).

2.2.4 Insulin resistance

Many studies have proved the importance of adipose tissue in glucose homeostasis, as it also stimulates glucose uptake in skeletal muscle and suppresses hepatic glucose production through the production of adipokines. Obesity can aggravate DM2 because it considerably increases proinflammatory cytokines secretion from the adipose tissue, such as tumor necrosis factor alpha (TNF-α), interleukins IL-1β, IL-18, IL-6, and nuclear factor kappa B (NF-κB) (TEIXEIRA; FARIA, 2021). Such factor can activate the expression of signaling inhibitors that can bind to the insulin receptors IR-1/2, preventing the insulin-induced tyrosine phosphorylation. In addition, excessive free fatty acids (FFA) and triglycerides that are ectopically deposited in muscle and liver, increase hepatic FFA oxidation, ROS levels and

activate the c-Jun N-terminal kinase pathway (JNK), which phosphorylates the threonine and serine residues of IR-1 and inhibits tyrosine phosphorylation of IR-1. This change prevents the coupling of IR-1 to the p85 subunit of PI3K. The alteration both by the excess of proinflammatory cytokines and by increased ROS levels, inhibits the activation of PI3K-AKT pathway, leading to insulin resistance (SAADELDEEN et al., 2020).

In addition to the common condition of insulin resistance, factors such as dehydration, fasting, increased metabolic demand, infection, and stress can also cause insulin resistance, but in a reversible manner, also by releasing proinflammatory cytokines, which inhibit the action of insulin. Mutations in the insulin signaling cascade may also be responsible for insulin resistance, such as type A insulin resistance syndrome, Rabson-Mendenhall syndrome, and leprechaunism. Abnormalities of mitochondrial function can also lead to insulin resistance; this occurs because there is a downregulation of the peroxisome proliferator activated receptor (PPAR) and co-activator 1-α (PGC-1α). The decreased expression of lipoprotein lipase (LPL) and PPARγ are responsible for a reduction in mitochondrial content. This decrease associated with lower levels of transcriptional genes that act on METC was observed in studies on obese patients (MASTROTOTARO; RODEN, 2021).

2.2.5 Oxidative stress effect on reducing insulin biosynthesis and secretion

Oxidative stress is directly related to the cause and progression of glucose toxicity and is associated with the molecular mechanisms of reduced insulin biosynthesis and secretion, as a result of the susceptibility of the pancreas and pancreatic islets to the redox imbalance installed, in addition to the smaller levels of antioxidant defenses, such as catalase and glutathione system (KANETO; MATSUOKA, 2012). Insulin biosynthesis is reduced as the pancreatic β cell is exposed to high concentrations of glucose. Oxidative stress has an inhibitory effect, like hyperglycemia, as it reduces the insulin gene's promoter activity, as well as the expression of its mRNA. Transcriptional factors that regulate the insulin gene are also reduced due to oxidative stress, such as the DNA binding capacity of pancreatic duodenal homeobox-1 (PDX-1) (KAWAHITO; KITAHATA; OSHITA, 2009), which has a direct role in the development of the pancreas, in differentiation, induction, maintenance and replacement of pancreatic β cells (KANETO; MATSUOKA, 2012). Similar changes occur with, MafA, a transcriptional factor that binds to the insulin promoter gene and is associated with the

regulation of insulin transcription in response to high plasma levels (HANG et al., 2014; ZHANG et al., 2005).

Therefore, hyperglycemia and oxidative stress consequently reduce the cellular expression of transcriptional factors PDX-1 and MafA, reducing insulin biosynthesis/secretion, which leads to hyperglycemia and thus ends up sustaining a vicious cycle. Oxidative stress markers such as 8-hydroxy-2-deoxyguanosine (8-OHdG) (AL-AUBAIDY; JELINEK, 2011; DONG et al., 2008), 4-hydroxy-2-nonenal (HNE) (PRADEEP et al., 2013; TOYOKUNI et al., 2000) and heme oxygenase-1 (HMOX1) (BAO et al., 2010; CHANDRAKUMAR et al., 2017) are increased in DM2 patients, showing that the generation of ROS is related to hyperglycemia, not only directly influencing secretion, but also involved in the adoption of alternative pathways that lead to hyperglycemia toxicity, as will be presented below.

2.2.6 Molecular pathways associated with oxidative stress and DM2 complications

Hyperglycemia is directly related to the production of ROS, and the reverse is also true, and both are related to DM2 complications, micro and macrovascular. When blood glucose is elevated, the glycolytic pathway, Krebs cycle, and METC also increase, causing an intensification in the difference in electrochemical potential, higher levels of ATP, and hyperpolarization of the METC. This increase in functioning promotes electron “escape” and partial oxygen reduction as already mentioned, resulting in higher levels of ROS, especially $\cdot O_2^-$ (BROWNLEE, 2001), as we already mentioned before. In addition, there is the adoption of alternative pathways, and the upregulated processes associated to the increase of ROS are considered the main factor for the activation and triggering of pathways. The alternative pathways: glycolytic, advanced glycation end products (AGEs); protein kinase C (PKC); aldose reductase (polyol pathway); and increased hexosamine flow (OKELEJI et al., 2021), are highly related to oxidative stress and the development of DM2 complications and will be better elucidated below.

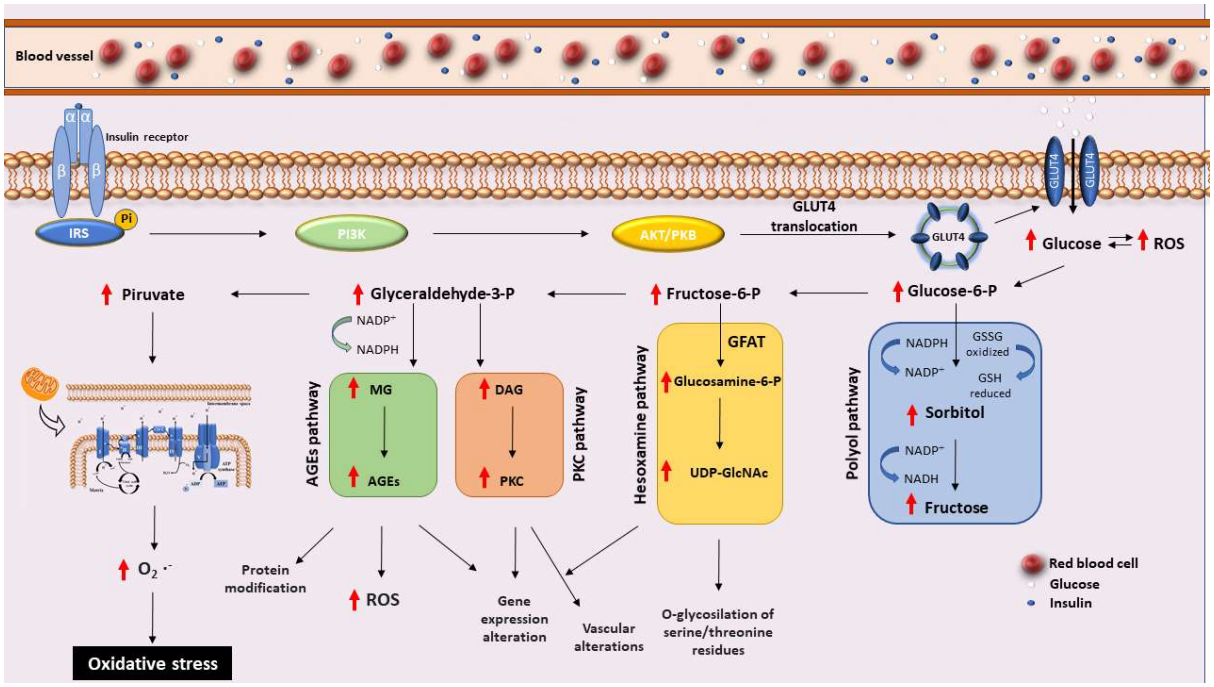


Figure 5: Intracellular mechanisms associated with hyperglycemia toxicity. Increased AGEs; activation of PKC pathway; and the aldose reductase pathway (polyol pathway); and increased hexosamine flow (MONTEIRO-ALFREDO, 2021 - Adapted from BROWNLEE, 2001, 2005).

Here, we outline the four main mechanisms resulting from the increased function of the glycolytic pathway that explain the toxicity of hyperglycemia and, as a result, the complications of DM2. According to Brownlee, the first of the four mechanisms discovered was the polyol pathway, years later the increase in the formation of advanced glycation end products (AGEs) was described, followed by the activation of the PKC isoforms and finally, the hexosamine pathway that results in the super modification of proteins (BROWNLEE, 2005). As a consequence, micro and macrovascular functions are affected, especially the functioning of the retina, glomeruli, skin, myocardium, and muscles, causing retinopathy, neuropathy, nephropathy in addition to cardiovascular diseases (CHAWLA; CHAWLA; JAGGI, 2016), and endothelial dysfunction (MAAMOUN et al., 2019). Several studies have been carried out, but there has always been an experimental failure regarding the discovery of a common element or a triggering factor for each of these pathways (BROWNLEE, 2005). To define a common element between the four mechanisms associated with glucose toxicity, Brownlee and collaborators in several studies have shown that oxidative stress and the consequent increased ROS levels are the main factor responsible for the cellular damage caused by the toxicity of hyperglycemia (DU et al., 2000; NISHIKAWA et al., 2000). Next, we briefly explain each pathway, as depicted in Figure 5, according to the production of each glycolytic intermediate.

2.2.6.1 Polyol pathway

The polyol pathway has the function of reducing toxic alcohol and aldehydes, and when under hyperglycemic conditions, it also converts excessive glucose into sorbitol, which is later converted into fructose (BROWNLEE, 2005). This pathway has a high activity regarding the consumption of glucose in the body, with a respective value of 30% (YAN, 2018). It is catalyzed by two enzymes, aldose reductase, which reduces glucose through the consumption of NADPH, and sorbitol dehydrogenase, which converts sorbitol to fructose, consuming NAD^+ and producing NADH (YAN, 2018). Besides the consumption of NADPH as a cofactor for glucose conversion, it is also essential for the intracellular regeneration of glutathione, through the reduction of glutathione.

This pathway is one of the main mechanisms that can explain the hyperglycemia toxicity since it is activated when glucose is in high concentrations. Aldose reductase is present in organs susceptible to the complications of DM2 and, in addition to the products originated by this pathway, it also generates cellular stress. Experiments in diabetic rats that had inhibited aldose reductase showed a prevention in the development of diabetic retinopathy (LORENZI, 2007). Hyperglycemia also promotes a reduction in the $NADPH/NADP^+$ ratio and an increase in the $NADH/NADP^+$ ratio, leading to the overload of the complex I of METC, increasing ROS generation (especially $\cdot O_2^-$ – the precursor of all ROS) (CHEN et al., 2019; SCHARTNER et al., 2018). Excess NADH also inhibits the glycolytic pathway and Krebs cycle, further increasing the activation of the polyol pathway (SCHARTNER et al., 2018; YAN, 2018). The decrease in NAD^+, on the other hand, promotes the inhibition of sirtuins, which are responsible for protein deacetylation, leading to protein over acetylation and less efficient glucose metabolism (MISHRA; DURAISAMY; KOWLURU, 2018).

The polyol pathway also increases the production of NO, sorbitol, fructose, and protein glycation. It is believed that sorbitol accumulation, as a consequence of sorbitol dehydrogenase decrease, alters osmotic pressure, acting as a main trigger factor for DM2 complications, nephro, retino and diabetic neuropathy (YAN, 2018). Regarding the DNA damage, it also upregulates the activity of poly (ADP-ribose) polymerase (PARP), an enzyme whose main function is to repair DNA damage. Upregulation depletes NAD^+ (which is also a substrate for PARP functioning) (YAN, 2018).

2.2.6.2 Hexosamine pathway

The hexosamine pathway is less activated by glycolysis (3%) than the other pathways (BUSE, 2006), and occurs after the conversion of glucose-6-phophate (G-6-P) to fructose-6-phosphate (F-6-P) with the aid of the enzyme glutamine-fructose-6-phosphate-amidotransferase (GFAT). GFAT converts F-6-P and glutamine into glucosamine-6-phosphate (GlcN-6-P) and glutamate, then into uridine 5'-diphospho-N-acetyl-D-glucosamine (UDP-GlcNAc) and CMP-sialic acid, which acts on protein glycosylation and energy metabolism (RUEGENBERG et al., 2020). The UDP-GlcNAc, the major end product of the hexosamine pathway, is an allosteric GFAT feedback inhibitor that regulates glucose flux in the pathway (BROWNLEE, 2005; BUSE, 2006).

Researchers believe that the hexosamine pathway acts as a cellular nutrient sensor and has an influence on the development of insulin resistance and vascular complications of DM2 (BUSE, 2006). The relationship of this pathway to insulin resistance was proposed by Marshal, in 1992, who carried out experiments with adipocytes isolated from rats. In this assay, the cells were pre-incubated with a high concentration of insulin and glucose, and the need for glutamine was proved for the development of insulin resistance, and consequently the involvement of the hexosamine pathway in the metabolic alteration (MARSHALL; BACOTE; TRAXINGER, 1991).

2.2.6.3 PKC pathway

The activation of PKC pathway is also a consequence of hyperglycemia and is the third alternative adopted pathway. Excessive glucose increases diacylglycerol (DAG) production, an important cofactor for the activation of several isoforms of PKC, mainly beta and delta (BROWNLEE, 2005). Their activation causes changes in gene expression, such as the change in the production of endothelial nitric oxide synthase (eNOS), which is reduced. In contrast, endothelin-1, an important vasoconstrictor, is increased. As a consequence, there are many pathological effects associated with the imbalance between eNOS and endothelin-1, mainly at the vascular level in cardiac, retinal, and renal tissue (BROWNLEE, 2005; KOYA; KING, 1998).

Inhibition of the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by the enzyme PARP and the consequent accumulation of its substrate glyceraldehyde-3-phosphate,

in addition to activating the PKC pathway by the accumulation of DAG, activates also another pro-oxidative pathway associated with the toxicity of hyperglycemia, the precursor of AGEs (especially the formation of methylglyoxal – MG), and seems to be fundamental for the relationship of oxidative stress and DM2 and its complications (IGHODARO, 2018). Additionally, the accumulation of upstream metabolites leads to an excessive stimulation of pro-oxidative pathways, such as hexosamine and polyol pathways (IGHODARO, 2018).

Hyperglycemia activates PKC and p38α mitogen-activated protein kinase (MAPK) in the diabetic retina and provokes the dephosphorylation of platelet-derived growth factor receptor-β, decreasing the downstream signaling of the receptor and leading to pericyte (cells that are found along the wall of capillaries, important for the formation of blood vessels (ATTWELL et al., 2016)) apoptosis (GIACCO; BROWNLEE, 2010). The increased activity of PKC pathway is associated to vascular complications (FORBES; COOPER, 2013), such as lower levels of NO production and seems to inhibit insulin-stimulated expression of eNOS, besides to increase the permeability-enhancing factor VEGF expression in smooth muscle cells. The PKC pathway was also shown to contribute to higher levels of transforming growth factor (TGF)-β_1, type IV collagen, and fibronectin in glomeruli of diabetic rats (GIACCO; BROWNLEE, 2010), and this seems to be mediated by NO inhibition (PUGLIESE et al., 1994). This pathway is also associated with overexpression of plasminogen activator inhibitor, fibrinolytic inhibitors and NF-κB activation of endothelial cultured cells (GIACCO; BROWNLEE, 2010), and it promotes the activation of angiotensin-converting enzyme (ACE), which is associated with apoptosis and necrosis of cardiomyocytes and endothelial cells (FORBES; COOPER, 2013).

2.2.6.4 Advanced glycation end products (AGEs) pathway

The non-enzymatic reaction between amino acid residues and carbonyl groups originated from reducing sugars, known as Maillard reaction, is divided into three phases: the first is the reaction between a sugar and an amino group and forms an unstable compound, the Schiff base, which will undergo a rearrangement, forming a more stable molecule, the Amadori product. Then, a dissociation of Amadori product occurs, forming dicarbonyl compounds, that is, the precursors of AGEs – glyoxal, MG, and deoxyglycosones. The last phase of glycation forms compounds with irreversible bonds, finally the AGEs. Proteins that suffer modifications by AGEs, lose their function and degrade into free AGEs, such glyoxal-lysine dimmer, methyl-

glyoxal-lysine dimer, N-ε-carboxy-methyl-lysine, and N-ε-carboxy-ethyl-lysine (KIKUCHI et al., 2003).

AGEs pathway is one of the main routes involved in DM2 complication etiology and progression (SINGH et al., 2014). Apparently, the AGE pathway can damage cells through three mechanisms. They are: 1) modification of intracellular proteins, especially the proteins involved in the regulation of transcription; 2) formation of AGEs to the extracellular matrix and consequent modification of the structure in the matrix of nearby molecules, altering cell signaling and causing its dysfunction; 3) precursors of AGEs diffused out of cells, also modifying proteins circulating in the blood, as is the case of albumin (BROWNLEE, 2005) and hemoglobin (MEERWALDT et al., 2008; TURK; MESIĆ; BENKO, 1998). The modification of circulating proteins can promote the binding and activation of AGEs receptors, causing the release of growth factors and proinflammatory cytokines, mainly causing vascular problems (BROWNLEE, 2005; MEERWALDT et al., 2008), conditions such as nephro-, neuro-, retino- and cardiomyopathy, and diseases as osteoporosis, rheumatoid arthritis, accelerated aging (SINGH et al., 2014) and cardiovascular diseases (FORBES; COOPER, 2013).

High levels of glucose trigger covalent adduct formation through non-enzymatic reactions between plasmatic proteins and glucose or glycating compounds originated from increased fatty acid oxidation or from glucose (GIACCO; BROWNLEE, 2010; SINGH et al., 2014). AGEs have some precursors (glyoxal, methylglyoxal, and 3-deoxyglucosone) that are increased in diabetic patients. Intracellular formation of AGE precursors can impair cell function through several mechanisms, such the modification of protein function; the extracellular matrix altered by AGE precursors interact anomalously with the matrix receptors expressed on the cell membrane surface; and last, the interaction between modified proteins binds to AGEs receptors (RAGE), like with vascular endothelial cells, macrophages, and vascular smooth muscle cells. RAGE activation leads to ROS generation, what activates nuclear factor-κB (NF-κB) (SINGH et al., 2014), provoking several pathological alterations, and immune and inflammatory responses (LIU et al., 2017).

2.2.7 Hyperglycemia toxicity and the consequent complications of DM2

Although blood with high concentration of glucose bathes all organs and tissues, only some tissues suffer from the consequences of the toxicity of hyperglycemia and are thus affected by the complications of DM2. Cells are normally able to reduce the transport of glucose

into the cell, when under hyperglycemic conditions, allowing the intracellular concentration to remain constant and healthy. However, endothelial and mesangial cells cannot make this regulation and become more susceptible (BROWNLEE, 2005). The persistent condition involving chronic hyperglycemia and oxidative stress lead to the development of DM2 complications, which are divided into micro and macrovascular. The microvascular are responsible for changes in small vessels and can be exemplified by changes that affect the retina, kidneys, and nervous, causing, respectively, diabetic retino, nephro, and neuropathy (FORBES; COOPER, 2013). The macrovascular damage to the arteries, causing cardiovascular disease that can result in acute myocardial infarction and angina pectoris, aortic sclerosis, and cerebrovascular disease, culminating in stroke (FORBES; COOPER, 2013; KAWAHITO; KITAHATA; OSHITA, 2009). Additionally, problems such as sexual dysfunction, dementia, depression (FORBES; COOPER, 2013), hypertension, soft tissue fibromatosis, osteopenia, lactic acidosis, and bacterial and viral infections, among others, can also be triggered (KAWAHITO; KITAHATA; OSHITA, 2009).

2.2.7.1 Microvascular complications

In general, DM2 complications are associated with redox imbalance, especially regarding the vascular function of patients. An example is the case of diabetic retinopathy, where according to a study carried out in diabetic rats, the high concentration of glucose in the retina is responsible for the elevation of persistent ROS, especially $\cdot O_2^-$ (KOWLURU; CHAN, 2007). The retina, for capturing more oxygen and suffering greater glucose oxidation, has a high susceptibility to oxidative stress, even higher than any other tissue (KANG; YANG, 2020; KOWLURU; CHAN, 2007). It was also shown that ROS accumulation is related to the cause, progression, and persistence of this condition, because even after the normalization of glucose levels in the body, the retinopathy reversal did not accompany the glycemic recovery, probably due to the higher ROS levels, but also as a side effect of hyperglycemia (KOWLURU; CHAN, 2007).

Nephropathy is the main cause of kidney failure, clinically characterized by the development of albuminuria (AMERICAN DIABETES ASSOCIATION, 2004), reduced glomerular filtration rate, and when untreated, uremia can be fatal (FORBES; COOPER, 2013; MOGENSEN; CHRISTENSEN; VITTINGHUS, 1983). Kidney disease can also trigger macrovascular complications such as cardiovascular, for example, causing hypertension and

stroke (CHRONIC KIDNEY DISEASE PROGNOSIS CONSORTIUM et al., 2010). Similarly, one-third of the diabetic population has neuropathy, a complication with painful symptoms (ABBOTT et al., 2011) with an estimated risk of limb amputation of 15%. It is believed that neuropathy is involved in somatic and autonomic damage of the peripheral nervous system, but it also affects the central nervous system and is a crucial factor for the aggravation of healing, cardiac and sexual dysfunction (FORBES; COOPER, 2013). In critical cases, such as advanced neuropathy, there is a loss of thermal perception that can evolve to degeneration of peripheral nerve fibers and sensory loss. A considerable number of patients with DM2 develop abnormal sensations such as hyperalgesia, spontaneous pain and paresthesia (OBROSOVA, 2009), and is also related to the development of depression, greatly reducing the quality of life (DZIEMIDOK; DĄBROWSKI; MAKARA-STUDZIŃSKA, 2016).

2.2.7.2. Macrovascular complications

The pathogenesis of macrovascular complications of DM2 is varied, but the main tissue affected is the endothelium. This occurs due to the inhibition of NO, responsible for promoting vasodilation (CADE, 2008). This inhibition is due to the increased production of the enzyme eNOS, responsible for the decrease in NO bioavailability. eNOS has its production increased also by the increase in the generation of ROS. Tissue plasminogen activator, which is activated by NO and has an anticoagulant function, is also reduced, consequently, its ability to reduce the adhesion of inflammatory cells to the endothelium is also inhibited. Insulin resistance increased free fatty acids, and activation of PKC pathway may also be potential inhibitors of eNOS. The increase in AGEs due to hyperglycemia also inhibits NO production. Additionally, there is an overproduction of vasoconstrictor factors, such as endothelin-1 (CADE, 2008; CHAN et al., 2000).

The macrovascular complications of DM2 have similar etiological factors, especially regarding the alternative pathways adopted in cases of chronic hyperglycemia and its toxicity (CADE, 2008). In general, DM2 is presented in the context of metabolic syndrome, associated with obesity, hyperlipidemia, hypertension, and coagulation disorders, which help in the development of cardiovascular diseases (FOWLER, 2008), the main cause of death in the world (WHO, 2021c). Cardiovascular diseases may also include cerebrovascular disease and peripheral artery disease, cardiomyopathy, coronary heart disease, arrhythmias, and sudden

death (VIIGIMAA et al., 2020). Importantly, DM2 patients have a 150% to 400% higher risk of having stroke episodes (FOWLER, 2008).

The main macrovascular alteration in DM2 is the atherosclerosis process, which occurs in response to chronic inflammation, lipid accumulation, and endothelial damage. It is believed that the increase in coagulability and platelet adhesion can aggravate the condition. As a result of this set of factors, the lipid-rich atherosclerotic lesion (atherosclerotic plaque) and a fibrous membrane is formed. Increased ROS/RNS and impaired NO formation can intensify this condition, helping to promote platelet aggregation, further increasing cardiovascular risk (FOWLER, 2008). When the atherosclerotic plaque becomes instable, it can detach from the vessel wall and cause stroke or a heart attack. DM2 is a risk factor for the development of several conditions, due to the chronicity of hyperglycemia. Interestingly, after the normalization of the other triggering factors mentioned above, DM2 still remains a critical and apparently independent factor (CADE, 2008), which supports the theory proposed by Brownlee regarding the adoption of alternative pathways for the chronic hyperglycemia and its consequent toxicity (GIACCO; BROWNLEE, 2010).

2.2.7.3. Acute DM2 complications

The acute complications of DM2 are already less specific, but very present in diabetic patients (FORBES; COOPER, 2013; KAWAHITO; KITAHATA; OSHITA, 2009). Increased glucose can lead to neurological, infectious, and cardiac problems, but can usually be alleviated with the treatment and reduction of hyperglycemia. Regarding the immune system, it becomes weaker, and the susceptibility to infectious also increases, especially in the surgical environment (FORBES; COOPER, 2013), and studies have reported a reduction in the immune response, especially the level of associated neutrophils to hyperglycemia, which induced considerable tolerance to lipopolysaccharides, a reduction in the production of cytokines and chemokines and an increase in the death of these defense cells in diabetic rats (KUWABARA et al., 2018).

2.2.8 Diabetes therapy

To maintain or restore the metabolic balance of patients, besides the adoption of a better lifestyle, with healthier diets, physical exercise, and an average of 7 hours of sleep (ranging

from 6 to 9 hours according to each person), in most cases, it is necessary the adoption of pharmacological therapies. The available medicines that are used in the clinic can be of several types, pharmaceutical forms, and different mechanisms of action (MARÍN-PEÑALVER et al., 2016). There are several pharmacological classes with different mechanisms of action, and here we bring a summary of them.

Biguanides (represented by Metformin) is the first pharmacological option used by physicians when contraindications are non-existent. It can alter the gut microbiota, activates AMP-activated protein kinase (AMPK) and inhibits hepatic gluconeogenesis (MARÍN-PEÑALVER et al., 2016). The activation of AMPK occurs through the phosphorylation of subunit α in Thr172, and indirectly the inhibition of Complex 1 in METC resulting in lower levels of cell energy that activates AMPK, restoring energetic homeostasis (ROGACKA; PIWKOWSKA, 2021). The class of sulfonylureas have a mechanism of action based on increased insulin secretion, regulated through ATP-sensitive potassium channels (KATP potassium channels) of pancreatic β cells, the insulin secretagogues of sulfonylureas, represented on the market by glibenclamide, for example (LEÃO et al., 2019; MARÍN-PEÑALVER et al., 2016).

Another class of drugs used to reduce hyperglycemia is the class of alpha-glucosidase inhibitors (represented by voglibose, miglitol, and acarbose). These drugs assist in postprandial hyperglycemia reduction, delaying glucose absorption through the competitive inhibition of alpha-glucosidase enzyme, that promotes carbohydrate hydrolysis (HOSSAIN et al., 2020). As they do not act on insulin release, alpha-glucosidase inhibitors do not cause hypoglycemia and do not affect body weight. The mechanism of action of these drugs is based on the postprandial reduction of hyperglycemia by causing late digestion and absorption of carbohydrates, by inhibiting (reversibly) the α-glucosidase hydrolase intestinalis enzymes that are linked to the membranes of the small intestine and are responsible for hydrolysis of oligosaccharide to monosaccharide (MARÍN-PEÑALVER et al., 2016).

The thiazolidinediones works by increasing insulin sensitivity in target tissues, such as adipose tissue, liver, and muscle (MARÍN-PEÑALVER et al., 2016). These drugs bind and activate PPARs and are potent insulin sensitizers and associated with two other receptors that work to regulate metabolic function (GELMAN; FEIGE; DESVERGNE, 2007). When PPAR are activated in the nervous system, it increases weight gain by stimulating feeding, in addition to improving the glycemic profile by preserving the function of the pancreatic β cell (MARÍN-PEÑALVER et al., 2016).

The dipeptidyl peptidase-4 inhibitors (IDPP4) acts by inhibiting the DPP4 enzyme, responsible for the degradation of incretins such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). These incretins are secreted by L cells in the intestine and induce insulin secretion and inhibit glucagon, thus iDPP4 increase the level of these hormones and consequently improves the glycemic profile (TAYLOR; LAM, 2020). This class includes linagliptin, alogliptin, sitaglipin, among others (MARÍN-PEÑALVER et al., 2016).

The sodium glucose co-transporter-2 inhibitors (ISGLT2) work in kidneys, inhibiting the reabsorption of glucose and increasing its excretion, reducing weight and blood pressure. SGLT2 is responsible for the reabsorption of 90% of glucose in the proximal tubule (MARÍN-PEÑALVER et al., 2016). Its mechanism depends on blood glucose concentration and is not related to insulin function, so the risks of patients presenting hypoglycemia by overstimulation or fatigue of β cells are low (KALRA, 2014), but in cases of patients with impaired renal function, its effects are reduced. Drugs in this class are: dapaglifozin, empagliflozin, and canagliflozin (MARÍN-PEÑALVER et al., 2016).

In addition to oral therapies, there are also injectable ones, such as the GLP-1 agonist (RA GLP-1), which mimics the incretins secreted by the body. They help in the secretion of insulin, reduce the release of glucagon, delay gastric emptying, reduce hunger and consequently also reduce caloric intake (RODRIGUES et al., 2020; TRUJILLO; NUFFER; ELLIS, 2015). They also have proven effects at the vascular level, increasing endothelial relaxation, protecting against endothelial dysfunction, and improving renal function with increased natriuresis and diuresis. Their use ends up being limited because they suffer rapid degradation by DPP4 (MARÍN-PEÑALVER et al., 2016). GLP-1 RA effect includes the reduction of fasting glycemia, HbA1c, body weight, and blood pressure (TRUJILLO; NUFFER; ELLIS, 2015).

Injectable insulin is also a therapeutic alternative for the treatment of DM2. The analogs of human insulin allow efficacy and efficiency and exist in the form of rapid action and prolonged release and are administered according to the needs of the patient and can have their different presentations combined. Insulin therapy can have some negative effects that limit its use, as it can cause weight gain and hypoglycemia, reducing patient quality of life, in addition to the low adherence to injections (MARÍN-PEÑALVER et al., 2016), but studies have been developed to simplify the use and administration, like insulin pumps, to ameliorate the therapy condition (GIUGLIANO et al., 2021).

Alternative therapies have been developed to aid in DM2 treatment, as is the case of metabolic surgery, applied to obese patients, which reduces glycemia and cardiovascular risk, and improves the lipid profile and consequently weight loss. Although there are no consistent theories that explain how the improvement of DM2 occurs (MARÍN-PEÑALVER et al., 2016), promising studies have been performed to better elucidate the effects of metabolic surgery, with results that suggest this therapy as one option, for presenting positive results regarding higher levels of hormones with anorectic effects and reduction of orexigenic hormones (EICKHOFF et al., 2016). Regarding therapies that may mean the future of DM2 treatment, there are studies in progress to produce GLP-1 RA (DAVIES et al., 2017) and insulin (ELDOR et al., 2013; KHEDKAR et al., 2010) as oral therapy, with promising data in human studies that make it possible to prospect these as a new therapeutic approach for the treatment of DM2.

2.3 Oxidative stress and cancer

According to the World Health Organization, one in every 6 deaths is caused by cancer, being the second leading cause of death worldwide, corresponding to 9.6 million cases. This disease has grown continuously throughout the population and has entailed high costs, both emotionally and financially, for patients and the health system, that has spent 1.16 trillion dollars on treatment in one year (WHO, 2021b). It is characterized by the uncontrolled growth of the body's cells, which impairs the functioning of normal cells and consequently alters the functioning of the body ('American Cancer Society', 2021).

2.3.1 Cancer pathophysiology

Cancer is developed due to genetic alterations that occur in the cell's DNA, specifically in proto-oncogenes, which under normal conditions are inactive. When activated, they become oncogenes and are responsible for making normal cells turn into cancer cells and by the modification of its cellular functions ('INCA - Instituto Nacional de Câncer', 2021). These genetic alterations that culminate in the progress of cancer are flaws developed in the body's repair mechanism, which are responsible for monitoring cell division and proliferation and must act from the moment that mutations or aberrations are identified. Since these protective mechanisms do not work properly, oncogenes and tumor suppressor genes are affected, leading to the development of metabolic alterations, abnormal cell division and proliferation, leading

to a carcinogenic process (MCADAM; BREM; KARRAN, 2016; TORGOVNICK; SCHUMACHER, 2015).

Cancer can be caused by genetic conditions (‘American Cancer Society’, 2021), but it is noteworthy that 80% to 90% of cases are caused by environmental or external conditions, such as exposure to causative agents (‘American Cancer Society’, 2021), such as the use of drugs related to the cancer treatment itself (KANEHIRA et al., 2016) and oxidative stress, which has a direct relation with the cause and progression of cancer (PARK et al., 2018).

2.3.2 Redox imbalance and cancer

DNA has its mechanisms to repair the damage suffered, which can originate from both endogenous and exogenous sources (Figure 6). Endogenous damages, represented, for example, by replication errors, are corrected by the system through the replacement of damaged or incompatible strands. However, in addition to replication errors, there are other spontaneous changes, such as non-enzymatic base hydrolysis and methylation, deamination, and the one believed to be the most critical source of damage, the ROS (HARTWIG; SCHWERDTLE, 2002). ROS act as toxic by-products of oxygen metabolism, especially the OH·, already mentioned in this review as one of the most reactive ROS existing, and thus, responsible for DNA damage (HARTWIG; SCHWERDTLE, 2002). The OH· radical has a half-time less than 1 ns, and as soon as it is formed, it already reacts in its own place of origin, and this can occur through several mechanisms, such as the decomposition of water by ionizing radiation, or photolytic decomposition of alkyl hydroperoxides, and when formed, the OH· radical reacts with DNA bases, inducing the production of damages bases or strand break (VALKO et al., 2006).

These injuries can occur in singles and double DNA strand breaks, cross-linking, and modification bases. This damage leads to changes and activation of mechanisms such as genomic instability, replication error, induction of the signal transduction pathway, and transcription arrest or induction (VALKO et al., 2006). In addition to the biological/pathological conditions of ROS production, and to the natural development of redox imbalance in cancer (RECZEK et al., 2017), chemotherapies can also promote redox imbalance, increasing ROS levels and reducing antioxidant defenses (ANDRISIC et al., 2018; MUT-SALUD et al., 2015). The oxidative stress caused becomes a side effect that generates damage

to various tissues (KANEHIRA et al., 2016) and greatly reduces the quality of life of patients (CAPPETTA et al., 2017).

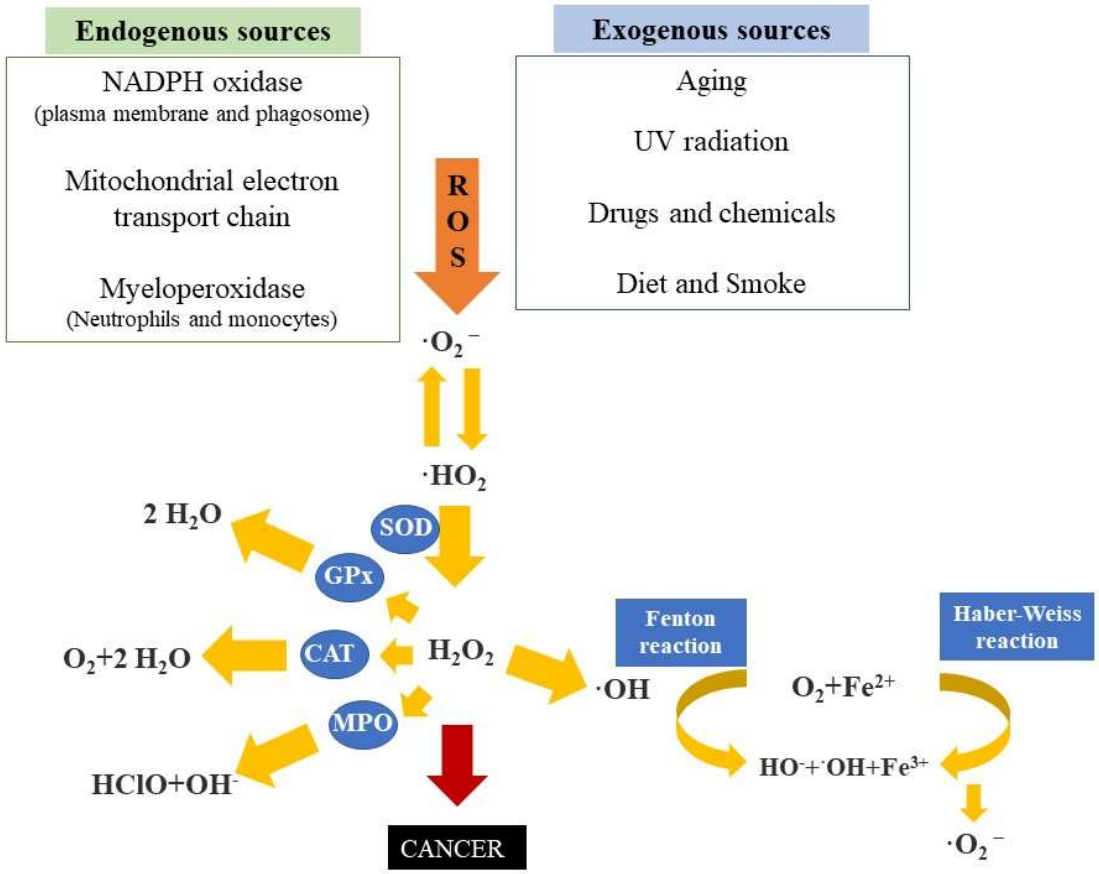


Figure 6. Implications of the redox imbalance on the development of cancer. Endogenous and exogenous sources of ROS (reactive oxygen species) can be varied, NADPH oxidase, mitochondrial electron transport chain, aging, UV radication, diets, drugs, among others. $\cdot O_2^-$ - superoxide, $\cdot HO_2$ - hydroperoxyl; H_2O2 - hydrogen peroxide; ·HO – hydroxyl; HClO - hypochlorous acid; (MONTEIRO-ALFREDO, 2021).

Among the chemotherapies greatly associated with oxidative stress, we highlight the alkylating agents, platinum coordination complexes (eg. cisplatin, carboplatin, and oxaliplatin), camptothecins (eg. topotecan and irinotecan), and anthracyclines (epirubicin and doxorubicin (CONKLIN, 2004), idarubicin and esorubicin) (LE BOT et al., 1988).

2.3.2 Doxorubicin (Dox)

Doxorubicin (Dox) (Figure 7), has the trade name ‘Adriamycin’ (GUVEN; SEVGILER; TASKIN, 2018) or ‘Adriblastina’ (‘Adriblastina® RD | Pfizer Brasil’, [s.d.]), is an antibiotic of the anthracycline class (KAVAZIS et al., 2017) non-selective class I (GUVEN; SEVGILER;

TASKIN, 2018) and recognized as a potent chemotherapeutic agent (CAI XINYONG et al., 2020), was first extracted from *Streptomyces peuceutius* var. caesius in the mid-1960s (BENJANUWATTRA et al., 2020). It is widely used in the treatment of various types of cancer, in solid tumors such as breast, lung (CAI XINYONG et al., 2020; ZHAO; ZHANG, 2017), gastric, ovarian and pancreatic cancer and hematologic malignances (BENJANUWATTRA et al., 2020), such as leukemias and lymphomas (GUVEN; SEVGILER; TASKIN, 2018).

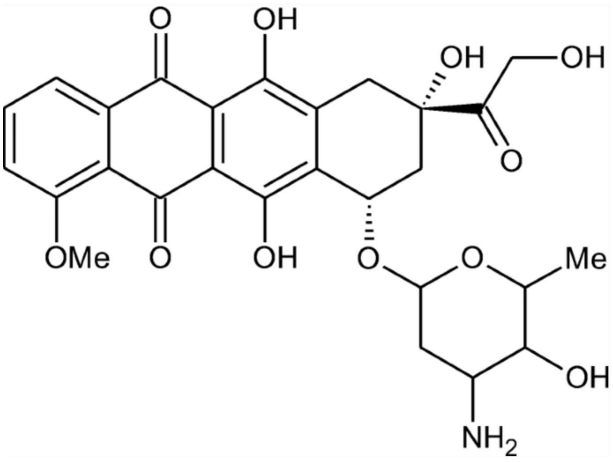


Figure 7. Doxorubicin chemical structure (BUTOWSKA et al., 2019).

2.3.2.1 Dox chemotherapy

Standard Dox treatment recommends intravenous administration with a range of 10 to 50 $mg.mL^{-1}$ (BARENHOLZ, 2012). Its peak of plasma concentration in humans are 5 to 15 $\mu mol.L^{-1}$ and a half-life in the range 20 to 30 h (BERTHIAUME; WALLACE, 2007). The binding rate to plasma proteins is low and the drug is rapidly distributed to the liver, heart, intestines, and kidneys, and its metabolism occurs in the liver with renal excretion (ZHOU et al., 2015).

2.3.2.2 Dox mechanism of action

The anticancer effect of Dox occurs through two distinct mechanisms; (I) Intercalation in the DNA with inhibits the enzyme Topoisomerase IIβ (TOPO2β), and changes in the structure of the chromatin; (II) Generation of ROS/RNS and oxidative damage to biomolecules, such as DNA and proteins (TAYMAZ-NIKEREL et al., 2018; THORN et al., 2011).

The mitochondria are the major ROS generator, and this occurs through its ROS producing enzymes. The production of ROS by Dox occurs through these mitochondrial enzymes, that promote the reduction of one electron from the quinone moiety in the ring C (H_2O_2 toxic by $\cdot O_2^-$) of Dox, transforming it into a semiquinone, an unstable metabolite that can react with oxygen, giving rise to a $\cdot O_2^-$ radical. This semiquinone can be neutralized and transformed by SOD into a stable H_2O_2 radical with low toxicity, or it can be transformed into other ROS/RNS that are part of the redox cycle. The problem is that OH· radicals can be generated from H_2O_2, which are highly reactive (CAPPETTA et al., 2017; KAVAZIS et al., 2017), causing lipid peroxidation and DNA and cell membrane damage, in addition to triggering cell death by apoptosis (THORN et al., 2011) and necrosis (CAPPETTA et al., 2017) due to cytochrome c release (CAI XINYONG et al., 2020).

These effects can also partly explain the cytotoxicity of the drug in non-target tissues, including cardiotoxicity, a recognized side effect of Dox chemotherapy (CAI XINYONG et al., 2020). This side effect reduces the patient quality of life and adherence to treatment (SHABALALA et al., 2017). Besides the increased ROS/RNS production, Dox cardiotoxicity is linked to the decrease of cardiac antioxidant defenses (ALANAZI et al., 2020), which leads to a pro-oxidative condition, as well as to mitochondrial dysfunction (WALLACE; SARDÃO; OLIVEIRA, 2020).

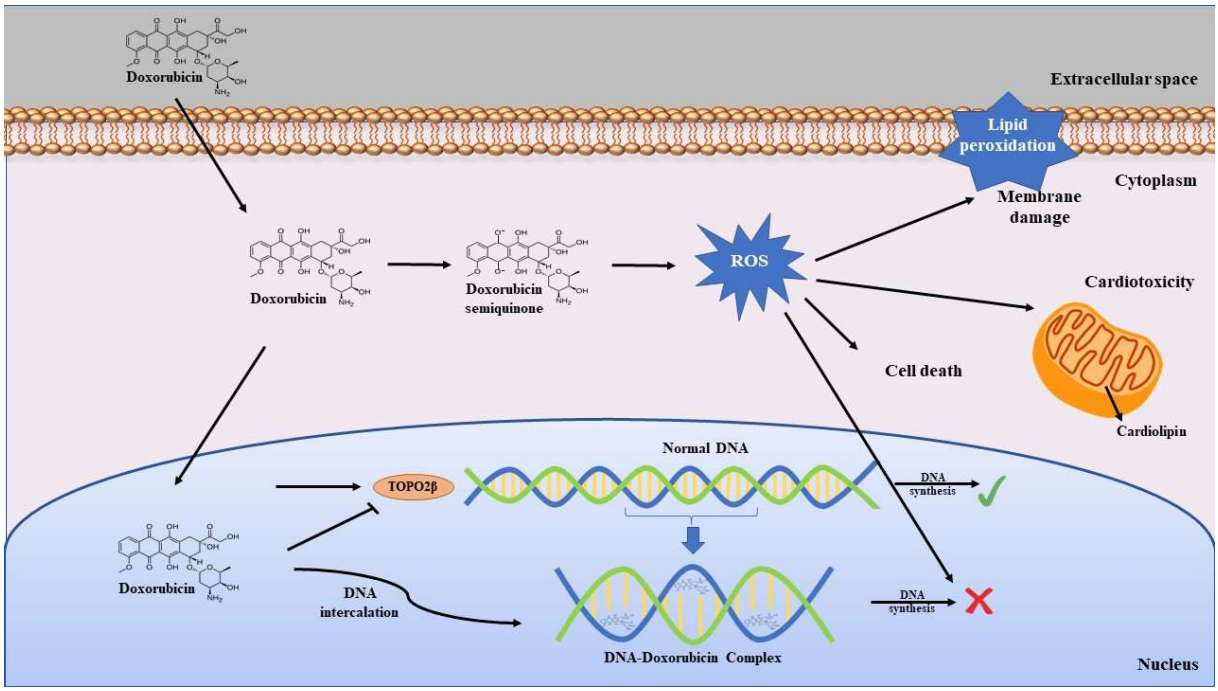


Figure 8. Main doxorubicin (Dox) mechanisms of action. Dox acts through the intercalation of the DNA, inhibiting the synthesis of the enzyme TOPO2β (topoisomerase 2β) and consequently preventing the synthesis of the DNA.

The generation of ROS (reactive oxygen species) also prevents DNA synthesis, induces damage to the cell membrane by promoting increased lipid peroxidation, and induces cell death. Additionally, as ROS have a greater specificity for cardiolipin present in the mitochondria of the cardiomyocytes, it accumulates in this organelle causing cardiotoxicity, which is further worsened by the fact that the heart has a lower expression of antioxidant enzymes (MONTEIRO-ALFREDO, 2021).

Like other drugs of the anthracycline class, Dox promotes the intercalation and disruption of DNA, mediated by inhibition of the enzyme TOPO2β. By inhibiting this enzyme that overwinds DNA during transcription, it prevents double strand recombination and consequently prevents its replication. There is also evidence related to the increased turnover of the nucleosome around Dox promoter, due to its intercalation activity (TAYMAZ-NIKEREL et al., 2018), in addition to another proposed mechanism that suggests the modification in gene and protein expression, besides the disruption of Ca^{2+} homeostasis (ZHAO; ZHANG, 2017), and mitochondrial dysfunction (GUVEN; SEVGILER; TASKIN, 2018).

2.3.2.3 Pathogenesis and mechanism of Dox cardiotoxicity

Although Dox is a remarkably effective anticancer agent, associated with this therapeutic effect, there are also side effects, and the main one is cardiotoxicity. Caused by cumulative dose-dependent administration, Dox-induced cardiotoxicity (THORN et al., 2011) promotes changes in myocardial structure and function, leading to severe cardiomyopathy, congestive heart failure (ZHAO; ZHANG, 2017), hypertension, arrhythmia, myocardial ischemia, pericardial disease, thromboembolic disease, and left ventricular systolic dysfunction (EWER; EWER, 2010). Patients treated with this chemotherapeutic may have a functional loss of cardiomyocytes and irreversible damage to the heart, which may result in a need of transplant or causing death (ZHAO; ZHANG, 2017). Cardiotoxicity is characterized by a substantial accumulation of ROS/RNS (CAPPETTA et al., 2017) and low protective effect of antioxidant enzymes (HALLIWELL; GUTTERIDGE, 1985). Cardiotoxicity is generally associated with ROS/RNS generation mechanism, since Dox effect on TOPO2β do not interfere in cardiotoxicity, as cardiomyocytes are generally not replicative (ZHAO; ZHANG, 2017). Despite this, there is increasing scientific evidence that cardiotoxicity is caused by other mechanisms in addition to the generation of ROS, such as senescence (CAPPETTA et al., 2017).

At the mechanistic level, in addition to the fact that the heart is an organ with less expression of antioxidant enzymes, the cardiotoxicity of anthracyclines is generated through the complex formed between Dox and cardiolipin (KAVAZIS et al., 2017), a heart-specific phospholipid rich in polyunsaturated fatty acids (MOBARAKI et al., 2017) Cardiolipins are located in the inner mitochondrial membrane, where anthracyclines binds to and accumulate, interrupting METC, further increasing the production of ROS. RNS are also related to cardiotoxicity, as studies suggest the existence of a crosstalk between the production of NO and Dox, showing that Dox binds to a specific domain of endothelial nitric oxide synthase (eNOS) and culminates in the accumulation of $\cdot O_2^-$. Peroxynitrite can also influence this process (MOBARAKI et al., 2017). The increase in ROS and its relationship with mitochondria has an important role in the context of chemotherapy performed with anthracyclines, as heart is an organ particularly rich in mitochondria (CAPPETTA et al., 2017).

2.4 The future of oxidative stress-related disease treatment

The research of agents against these diseases with fewer side effects, more affordable prices, and a considerable pharmacological effect, has a great importance in terms of public health, and is a challenge for science. In this context, medicinal plants are an important target, especially phenolic compounds, which are substances with strong antioxidant potential with the ability to regulate glucose metabolism (MARÍN-PEÑALVER et al., 2016) through various metabolic pathways, such as improving insulin secretion/sensitization, increasing glucose uptake, helping in the restoration of β cell integrity in the case of DM2 complications (KAWAHITO; KITAHATA; OSHITA, 2009; MARÍN-PEÑALVER et al., 2016). They can also act as anticancer therapies (JAFARI; SAEIDNIA; ABDOLLAHI, 2014) by stopping the cell cycle (JAFARI; SAEIDNIA; ABDOLLAHI, 2014), inhibiting or reducing cell adhesion, migration, proliferation and differentiation or inhibiting DNA binding in case of cancer treatment (HUANG; CAI; ZHANG, 2009), in addition to the inhibitory mechanism of several pathways that act in both conditions.

Thus, medicinal plants are an alternative to help restore/maintain the redox balance (MONTEIRO-ALFREDO et al., 2020a; YAN, 2018) and consequently can act against oxidative stress-related diseases. In this subtopic, we explore in more detail the medicinal plants as a therapeutic alternative, highlighting Brazilian biodiversity, the Cerrado plants rich in phenolic compounds.

2.4.1 Brazilian biodiversity

The continental proportions and the 8.5 million km2 that make up almost half of South America, allow Brazil to have several climatic zones and ecological variations, which are formed in biogeographic zones, better known as biomes (FERNANDES et al., 2016). They range from the Amazon and Atlantic Forest, Savannah, Pantanal to the Cerrado, which support a wide variety of fauna and flora (FERNANDES et al., 2016), corresponding to 20% of the total number of species worldwide (GALÚCIO et al., 2019), and including 40,000 phytochemicals-rich plant species (CARVALHO; CONTE-JUNIOR, 2021; OLIVEIRA et al., 2012).

Among the different Brazilian biomes, the Cerrado occupies 21% of the national territory and possesses 5% of the world's biodiversity, what makes Cerrado the second largest biome in Brazil (RIBEIRO NETO et al., 2020). Its area extends across the states of *Mato Grosso, Mato Grosso do Sul, Distrito Federal, Goiás, Paraná, Minas Gerais, Bahia, São Paulo, Tocantis, Rondônia*, *Piauí* and *Maranhão*, which has in its subsoil the largest hydrographic basins of the continent – *Bacia do São Francisco, do Rio da Prata* and *Amazônia/Tocantins.* As a result, this is a considerable aquifer reservoir supporting biodiversity (MMA, 2020).

Cerrado possesses several endemic species, which makes it as being recognized as the largest savanna worldwide, with 160,000 species of plants, fungi, and animals (RATTER; RIBEIRO; BRIDGEWATER, 1997). In this context of biodiversity, we highlight the bioactive compounds of plants, which are potential therapeutic agents that contribute to the maintenance and treatment of chronic diseases (OLIVEIRA et al., 2012), among them cancer (GALÚCIO et al., 2019) and DM2 (CARVALHO; CONTE-JUNIOR, 2021) and in helping to maintain the body's redox balance (DOS REIS et al., 2020; SERAGLIO et al., 2017). Besides an important relationship with bioenergy production through eucalyptus and sugarcane, this biome is also responsible for 70% of Brazilian agricultural production (GOMES et al., 2019).

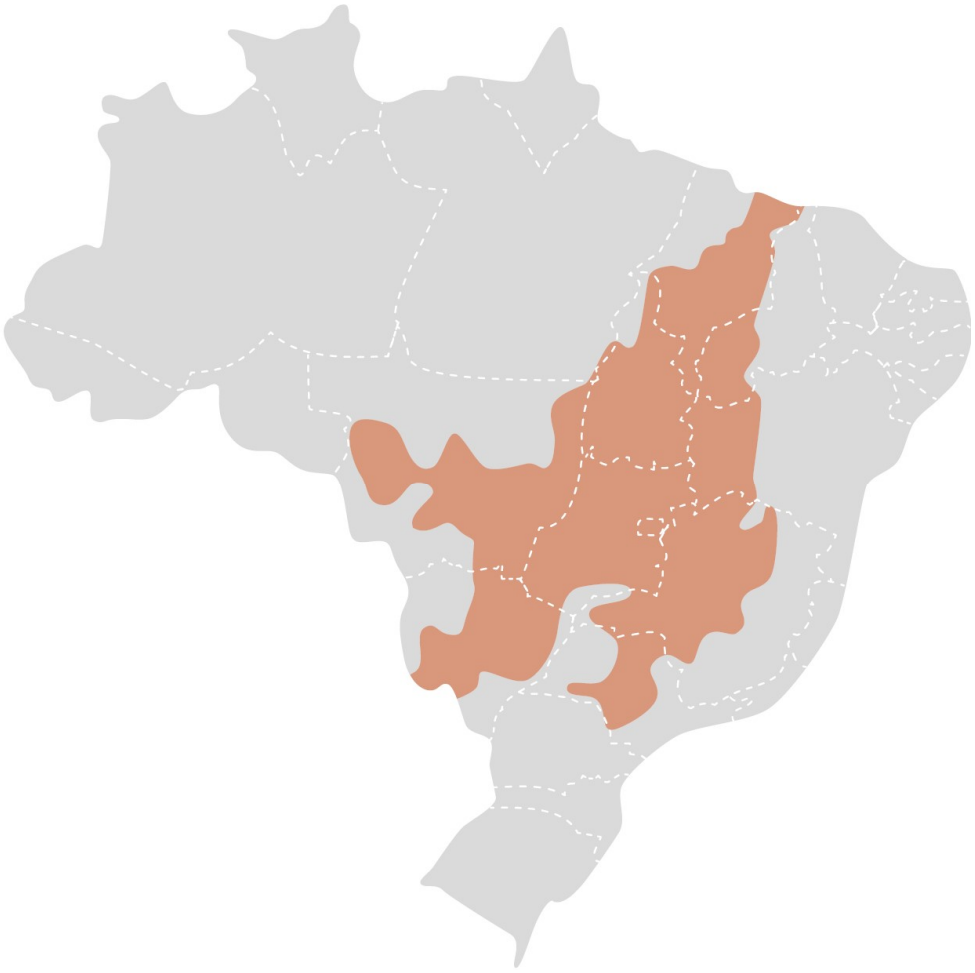


Figure 9. Map of Brazilian Cerrado (NATIONAL GEOGRAPHIC, 2020).

2.4.2 Medicinal plants

The definition of a medicinal plant applies to any plant with substances used for therapeutic purposes or in the development of new drugs (SOFOWORA; OGUNBODEDE; ONAYADE, 2013). It is estimated that between 65% to 80% of the world population uses folk medicine or some ethnopharmacological practice, or directly depends on some popular practice for the treatment of diseases or health maintenance (WHO, 2011). More than 50% of the new drugs currently developed are derived from medicinal plants or use medicinal plants as raw material (TENG; SHEN, 2015) and these numbers represent only 15% of the 300,000 species of existing plants in the world (DE LUCA et al., 2012). From the economic point-of-view, the plant global market is estimated in $83 billion (PALHARES et al., 2015) to $100 billion per year (SOFOWORA; OGUNBODEDE; ONAYADE, 2013).

Plants are also a great source of potential chemical compounds that are available for the development of alternative therapies. Due to their extensive, there are some documents

published by WHO (WHO, 1999, 2003, 2007, 2009) and by the Brazilian government (‘Ministério da Saúde’, 2008), which contains lists of recognized species with medicinal properties well established. In addition, policies that encourage the use of medicinal plants in Brazil, namely the called ‘National Policy on Medicinal Plants and Herbal Medicines’ (from the Portuguese: *Programa Nacional de Plantas Medicinais e Fitoterápicos*), aims to implement actions to promote quality of life for the population using strategies to improve the population’s access to medicinal plants (PNPMF, 2009). *Mikania glomerata*, *Maytenus illicifolia L.*, *Miracrondruon unrudeuva* and *Lippia sidoes Cham.*, are some examples of plants included in the ‘National Policy on Medicinal Plants and Herbal Medicines’, which has studies based on ethnopharmacological practices and information about the drugs, which reduces time and investments, facilitating its use (PNPMF, 2009).

Brazilian biodiversity has high economic value regarding dietary and herbal products, due to the variety of primary and secondary plant metabolites (CARVALHO; CONTE-JUNIOR, 2021). Secondary metabolites increase the plant chemical complexity and are directly linked to their biological activity. They include tannins, coumarins, terpenoids, cardiotonic glycosides, anthocyanins, saponins, phenolic compounds, and flavonoids (TAFOLLA-ARELLANO et al., 2013). The pharmacological potential of these plants may help in reestablishing the body's redox balance in the treatment of chronic diseases (DOS REIS et al., 2020; SERAGLIO et al., 2017). In the specific case of phenolic compounds and flavonoids, they have antioxidant, anti-inflammatory and anti-cancer activities (ARAÚJO, J, 2008).

2.4.3 Secondary plant metabolites - phenolic compounds

Phenolic compounds are a group of secondary metabolites that have in common an aromatic ring attached to one or more hydroxyl groups. Naturally, there are around 8,000 phenolic plants and, of these, about half are flavonoids (SULAIMAN; BALACHANDRAN, 2012). In addition, there are some other subclasses of secondary metabolites that belong to the phenolic compounds: lignans, tannins, stilbenes, which have carbon in their chemical structure that determine the complexity of its structure and consequently its biological activity (CHEN et al., 2021).

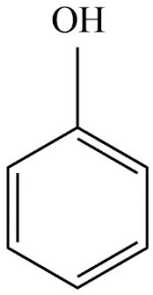


Figure 10. Basic phenol structure (YAN et al., 2021, p. 2)

Flavonoids (Figure 11) contain two benzene rings separated by a propane (SULAIMAN; BALACHANDRAN, 2012) and the rings are A, C, and B in C6-C3-C6 (DAI; MUMPER, 2010). Within the group of flavonoids, there are 6 subgroups that include flavanones, flavones, flavonols, flavanols, isoflavones, and anthocyanins, and their group operates in the oxidation state of the central ring "C". The differences of each subgroup occur according to the degree of hydroxylation, methylation, glycosylation, and prenylation that each molecule undergoes. Catechins, naringenin, quercetin, rutin are examples of flavonoids (ALARA; ABDURAHMAN; UKAEGBU, 2021).

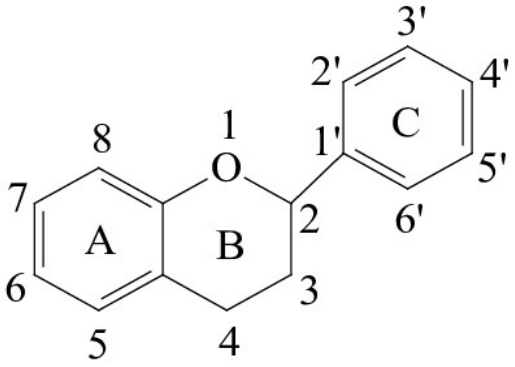


Figure 11. Basic flavonoid structure (KUMAR; PANDEY, 2013).

There are 2 classes of phenolics (Figure 12), derived from benzoic acid (gallic acid) and cinnamic acid, with examples as ferulic, caffeic, and coumaric acids (DAI; MUMPER, 2010). Tannins are divided in two classes, the hydrolysable and the condensed ones. The hydrolysable tannins contain a central glucose core in an esterified form with gallic acid; the condensed are either polymers of flavan-3-ol or oligomers via the interflavan carbon bond. The condensed tannins are known as proanthocyanidins, because when heated in an acid alcohol solution, they

turn into anthocyanidins through a process of acid-catalyzed oxidation (NAUMANN et al., 2017).

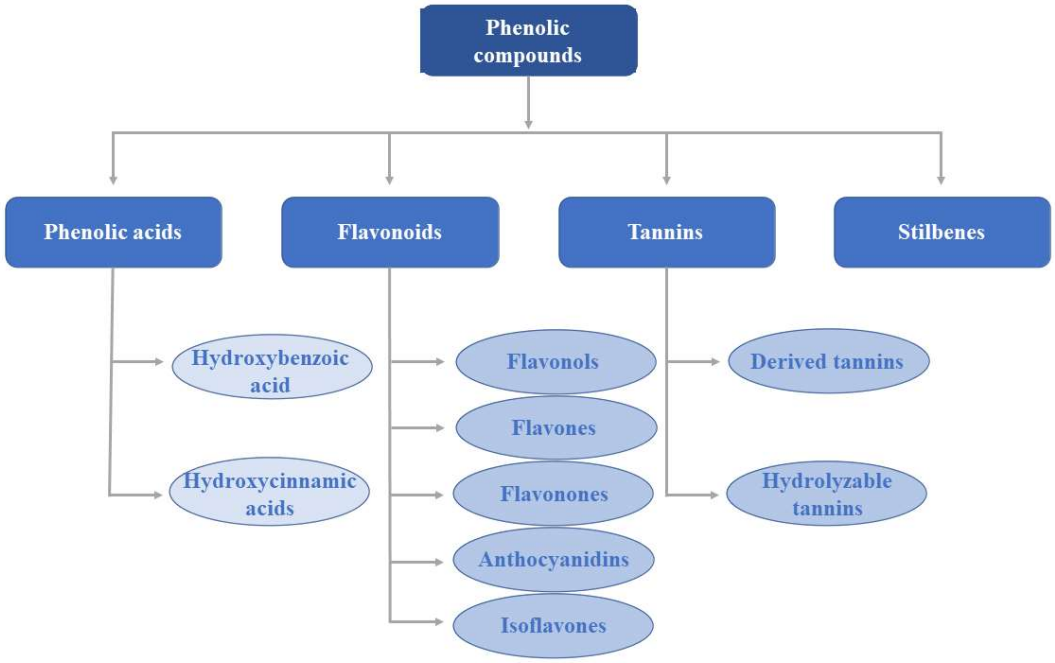
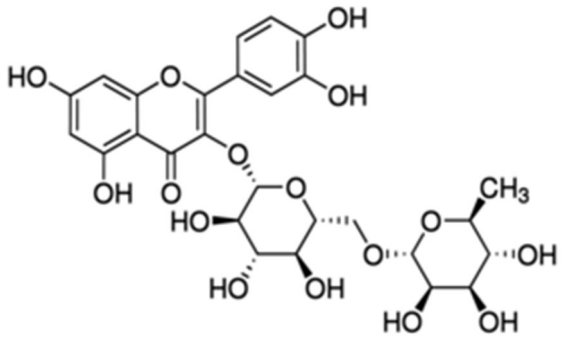
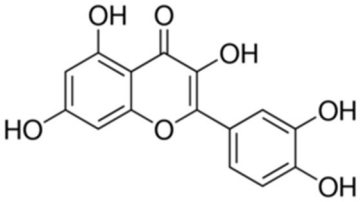
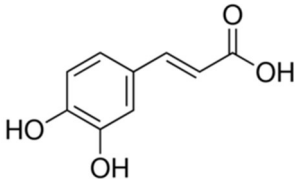
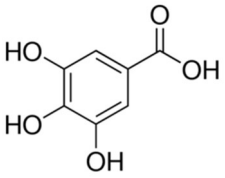
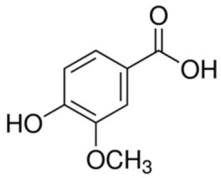
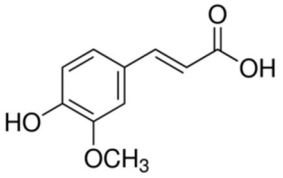


Figure 12. Main subclasses of the phenolic compounds (ALARA; ABDURAHMAN; UKAEGBU, 2021).

Phenolic compounds (Figure 12) are found in fruits, vegetables, cereals, and beverages (tea and wine), being responsible for the organoleptic characteristics, mainly color and flavor (ALARA; ABDURAHMAN; UKAEGBU, 2021). They have a wide range of therapeutic activities, including regulation of angiogenesis, apoptosis, cellular proliferation, and cell cycle (MAYA-CANO; ARANGO-VARELA; SANTA-GONZALEZ, 2021). They also have antibacterial, cardioprotective, anti-inflammatory, immune system stimulator, UV radiation protection (TUNGMUNNITHUM et al., 2018), antimutagenic, anticarcinogenic and antioxidant activities (MARINOVA; RIBAROVA; ATANASSOVA, 2005).

The antioxidant properties that characterize phenolic compounds for being the largest group of phytochemical compounds with antioxidant potential are based on the presence of phenolic hydroxyl groups attached to ring structures which act as reducing agents, such as metal chelators, hydrogen donators, $\cdot O_2^-$ scavenger, singlet oxygen quenchers and activate antioxidant enzymes (CAROCHO; FERREIRA, 2013; OKPUZOR, J. et al., 2009). Below are represented the molecules of the phenolic compounds (gallic, ferulic, caffeic, vanillic acid) and flavonoids (rutin and quercetin) of interest in this work.

Table 1: Nomenclature and general chemical structure of flavonoids and phenolic compounds.

Chemical compound	Molecule
Rutin	
Quercetin	
Caffeic acid	
Gallic acid	
Vanillic acid	
Ferulic acid	

(SIGMA-ALDRICH; MERCK, 2021).

2.4.4 Phenolic compounds and oxidative stress detoxification pathways - a therapeutic strategy for the activation of key pathways

Phenolic compounds are considered an important therapeutic strategy in maintaining the body's redox balance, in addition to being used in the treatment of oxidative stress-associated chronic diseases (MONTEIRO-ALFREDO et al., 2020a), such as neurodegenerative disorders, metabolic syndrome, DM2 and cancer (ARRUDA et al., 2020; SANTOS-BUELGA et al., 2019). In this context, the investigation of the mechanisms of action of phenolic compound-derived products becomes important for the treatment of diseases and maintenance of health. The presence of these secondary metabolites in vegetables gives their beneficial properties, being consumed as foods or beverages, they can also be used for the development of drugs, and several studies are being developed with plants. Supporting this information, *Lythrum salicaria* L. (Lythraceae), for example, is considered a promising source of phenolic compounds that modulate redox imbalance, as it has a considerable antioxidant protective effect, proven through the reduction of DNA damage induced by hydroxyl and peroxyl radicals, without presenting toxicity in normal cells (SREĆKOVIĆ et al., 2020). The phenolic compounds present in *Vaccinium corymbosum* L. also showed important protection against the oxidative damage of ROS induced by H_2O_2, lower levels of malondialdehyde (MDA), and good inhibition of 1,1-diphenyl-2-picrylhydrazine (DPPH) and the ferric reducing antioxidant power test (FRAP) (MANQUIÁN-CERDA et al., 2018). Another study that used similar parameters to evaluate the antioxidant effect was carried out in *Triplaris gardneriana* and revealed a protective effect with lower levels of generated MDA, good iron chelating effect and DPPH radical scavenging activity (DE ALMEIDA et al., 2017), *Fraxinus angustifolia* (KASMI et al., 2021), and *Euterpe oleracea* Mart. (MELO et al., 2021) also showed the ability to scavenge DPPH and ABTS free radicals.

The association of the antioxidant effect and other therapeutic properties of phenolic compounds were also evidenced in some plants, as is the case of *Rumex dentatus* L., which showed free radical scavenging capacity and a reduction in glycemia, hepatic injury and improved carbohydrate metabolism by upregulating PPARγ in diabetic rats (ELSAYED et al., 2020). Liver damage prevention associated to the protective effect and increased expression of antioxidant enzymes was also evidenced in *Bridelia tomentosa* (MONDAL et al., 2021). Studies performed to analyze oxygen radical absorbance capacity (ORAC) revealed the antioxidant ability of phenolic compounds found in extra virgin olive oils, associated with lower levels of fluorescence from the DCFH-DA probe in NIH-3T3 cell line (PRESTI et al., 2017). Similar effects of reducing the fluorescence intensity of DCFH-DA and preventing oxidative damage were reported in an assay carried out with *Suaeda monoica*, which also showed a

reduction in methylglyoxal-induced toxicity in human endothelial cells (HUVEC) and inhibition of caspases 3 and 7 (PARVEZ et al., 2021). Phenolic compounds of *Vaccinium* spp berries presented interesting effects in an experiment carried out in cell culture of skin cells, with cell cycle and proliferation inhibition, and reduction of malignant cells, macromolecules oxidation, pro-inflammatory cytokines and oxidative stress (MAYA-CANO; ARANGO-VARELA; SANTA-GONZALEZ, 2021). *Butia catarinensis*, *Butia eriospatha* and *Opuntia elata*, which showed antioxidant effects *in vitro* against hydrogen peroxide H_2O_2, OH· and ROO· and ABTS radicals, and showed *in vivo* protection of nematodes *Caenorhabditis elegans* induced by NaCl and evaluated un relation to the fluorescence of DFCH-DA (CAMBOIM ROCKETT et al., 2020).

In addition to the plants mentioned above, which have phenolic compounds and therapeutic properties, the same as those mentioned in this review, we sought to analyze the current literature with published studies that report plants derived from the Brazilian Cerrado that have similar characteristics to those mentioned above. For this, a literature search was performed in PubMed and Science Direct (until September 2021). The search terms used were 'phenolic compounds', 'Cerrado plant', 'antioxidant', 'diabetes', and 'cancer'. The obtained data are represented in Table 2.

Table 2: Phytochemical constitution and biological properties of medicinal plants from the Brazilian Cerrado.

Medicinal plant	Phytochemical constituents	Biological properties	Reference
Acrocomia aculeata	Gallic, vanillic, caffeic, and ferulic acid, rutin, quercetin, campesterol, stigmasterol, *β*-sitosterol, lupeol, and lupeol acetate	*In* vitro and *in vivo* antioxidant activity, hypoglycemic and hypotriglyceridemic effect	(MONTEIRO-ALFREDO et al., 2020a, 2021)
Alibertia edulis	Caffeic acid, quercetin 3-rhamnosyl-(1 → 6)-galactoside and iridois ioxide	Hypoglycemiant effect, protection against hemolysis and oxidative stress	(FIGUEIREDO DE SANTANA AQUINO et al., 2020)

Annona crassiflora	Epicatechin and quercetin	Antioxidant, antiproliferative and wound healing activity	(PRADO et al., 2020)
Annona muricata	Total phenolic compounds, flavonoids and proanthocyanidins (total quantification)	Antioxidant activity, *in vitro* antidiabetic and inhibitory potential against α-amylase, α-glucosidase, lipase, non-enzymatic glycation, and lipid peroxidation	(JUSTINO et al., 2018)
Bactris setosa	Phenolic compounds (anthocyanins and non-anthocyanin phenolic compounds) and carotenoids	Oxidative and nitrosative protection	(BOEING et al., 2017)
Banisteriopsis argyrophylla	Catechin, procyanidins, glycosylated flavonoids, kaempferol, and megastigmane glucosides	α-amylase, α-glucosidase, lipase, and glycation inhibitors antidiabetic and antioxidant activity	(QUARESMA et al., 2020)
Byrsonima verbascifolia	Resveratrol and ferulic acid	Antimutagenic, antigenotoxic and antioxidant activity	(MALTA et al., 2012)
Campomanesia cambessedeana	Catechin, ethyl gallate and propyl gallate	Antimutagenic, antigenotoxic and antioxidant activity	(MALTA et al., 2012)
Caryocar brasiliense	Gallic acid, quinic acid, quercetin, and quercetin 3-O-arabinos	Antioxidant activity	(ROESLER et al., 2008)

Cedrela odorata	Gallic acid, catechin and gallocatechin	Hyperglycemia reduction and antioxidant activity *in vivo*	(GIORDANI et al., 2015)
Dipteryx alata	Gallic acid and its derivatives, such as gallic acid esters and gallotannins	Antioxidant and antiproliferative activity	(OLIVEIRA-ALVES et al., 2020)
Dipteryx alata	p-Coumaric, ellagic, caffeic, ferulic, and gallic acid and hydroxybenzoic, catechin and epicatechin	Antioxidant activity	(LEMOS et al., 2012)
Dipteryx alata	Phenolic compounds (total quantification)	*In vivo* antioxidant activity	(SIQUEIRA et al., 2012)
Dipteryx alata	Phenols, terpenes, fatty acid derivatives, vitamins, and a carboxylic acid.	Antioxidant activity and *Caenorhabditis elegans* life expectancy increase	(LEITE et al., 2020)
Eschweilera nanat	Rutin and hyperoside	Antioxidant activity	(OUTUKI et al., 2016)
Eugenia dysenterica	Proanthocyanidins, flavonoids, phenolic acids, quercetin, kaempferol derivatives, free and total ellagic acid	Antioxidant activity, pancreatic lipase inhibition, body weight and fat mass gain, hyperglycemia and dyslipidemia attenuation and fecal triglycerides excretion improved	(DONADO-PESTANA; BELCHIOR; GENOVESE, 2015)

Eugenia dysenterica	Phenolic compounds (total quantification), myricetin, quercetin and kaempferol	Antioxidant, antiproliferative and antimutagenic potential	(NERI-NUMA et al., 2013)
Guazuma ulmifolia	Flavan-3-ol-derived flavonoids, including monomers and dimers, condensed tannins, and glycosylated flavonoids	*In* vitro and *in vivo* antioxidant activity	(DOS SANTOS et al., 2018a)
Hancornia speciosa	Phenolic compounds (total quantification)	Antioxidant activity	(DE LIMA et al., 2015)
Hymenaea stignocarpa	Caffeic acid, kaempferol, quercetin-3-rutinoside and quercetin-3-rhamnoside	α-amylase and α-glucosidase inhibition, glycemic profile improved	(SILVA et al., 2019)
Hyptis Jacq.	Phenolic acids, flavonoids, chlorogenic acid and cinnamic acid derivatives, rosmarinic acid and total phenolic compounds (total quantification)	Antioxidant activity	(DOS SANTOS et al., 2018b)
Mauritia flexuosa	Total phenolic compounds and β-carotene (total quantification)	Antioxidant activity	(CÂNDIDO; SILVA; AGOSTINI-COSTA, 2015)
Mauritiella armata	Palmitic, estearic, oleic, linoleic, linolenic acid, tocopherol, and α-tocopherol	Antioxidant activity	(DE SOUZA et al., 2021)

Myrcia bella	Flavonoids and phenolic acids derivatives	Antimutagenic and antioxidant activity	(SERPELONI et al., 2015)
Psidium cattleianum	Epicatechin, gallic, coumaric, and ferulic acid, myricetin and quercetin	Antioxidant and antimicrobial activity and antiproliferative effect on human cancer cells	(MEDINA et al., 2011)
Schinus terebinthifolius Raddi	*O*-glycosylated flavonols, gallotannins and gallic acid along with its derivatives	Antioxidant, antidiabetic and antiproliferative activities	(DOS SANTOS DA ROCHA et al., 2019; OLINTO et al., 2020)
Sterculia striata	Oleic acid, phytosterols β-sitosterol, stigmasteroland, campesterol γ-, δ-, α- and β-tocopherol, ellagic, ferulic, methoxyphenylacetic and protocatechuic acids	Antioxidant activity	(DE BRITTO POLICARPI et al., 2018)
Solanum lycocarpu	24 phenolic compounds (see ref.)	Antioxidant activity	(PEREIRA et al., 2019)
Vochysiaceae species	Polyphenols, such as flavonoids and condensed tannins	Antioxidant and inhibitory potential against human α-amylase and protein glycation	(FRANCO et al., 2019)
Senna velutina	21 compounds (see ref.)	*In vitro* and *in vivo* antitumor effects	(CASTRO et al., 2019)

2.4.5 Nuclear factor erythroid 2 (NFE2)-related factor 2 (NRF2)

The organism has mechanisms that act in the regulation and maintenance of the redox balance, and one of these mechanisms is the nuclear factor erythroid 2 (NFE2)-related factor 2 (NRF2), a cap 'n' collar subfamily member of the basic region leucine zipper transcription factor (MA, 2013). NRF2, discovered in 1994 (CHEN; MALTAGLIATI, 2018), is a key transcription factor that regulates several antioxidant mechanisms, cellular homeostasis, detoxification genes (PENG et al., 2021), and acts as a cytoprotective regulator (LAZARO et al., 2018). NRF2 mRNA is widely expressed and independently of inducers. Its modulation occurs in two ways, activated by inducers and under stress conditions and suppressed by physiological state and suppressor agents (iNRF2) (MA, 2013). Under the canonical pathway, it is negatively regulated by a cytosolic hijacking complex, Kelch-like ECH-associated protein 1 (Keap1, iNRF2) and supports the ubiquitination and degradation of NRF2 in a constant way, to keep NRF2 concentrations low under physiological conditions.

Under oxidative stress conditions, the canonic pathway, NRF2 is phosphorylated, dissociated from Keap1 in the cytoplasm and translocated to the nucleus (ERLANK et al., 2011; FENG et al., 2018), where binds to antioxidant response elements (AREs) transcription detoxifying genes (LAZARO et al., 2018), being responsible for the expression of phase-2 detoxifying enzymes, such as GPx, GST, CAT (ERLANK et al., 2011), HMOX1, among others (Figure 13) (LAZARO et al., 2018). The non-canonical pathway of NRF2 is associated with autophagy. It is a cysteine-independent mechanism, and sequesters Keap1 for degradation by autophagy, so that NRF2 stabilizes and transactivates its dependent genes. The autophagy pathway has the function of removing damaged organelles and protein aggregates that occur through lysosomal degradation so that the homeostasis of the cell and the metabolic processes involved are maintained (LAZARO et al., 2018).

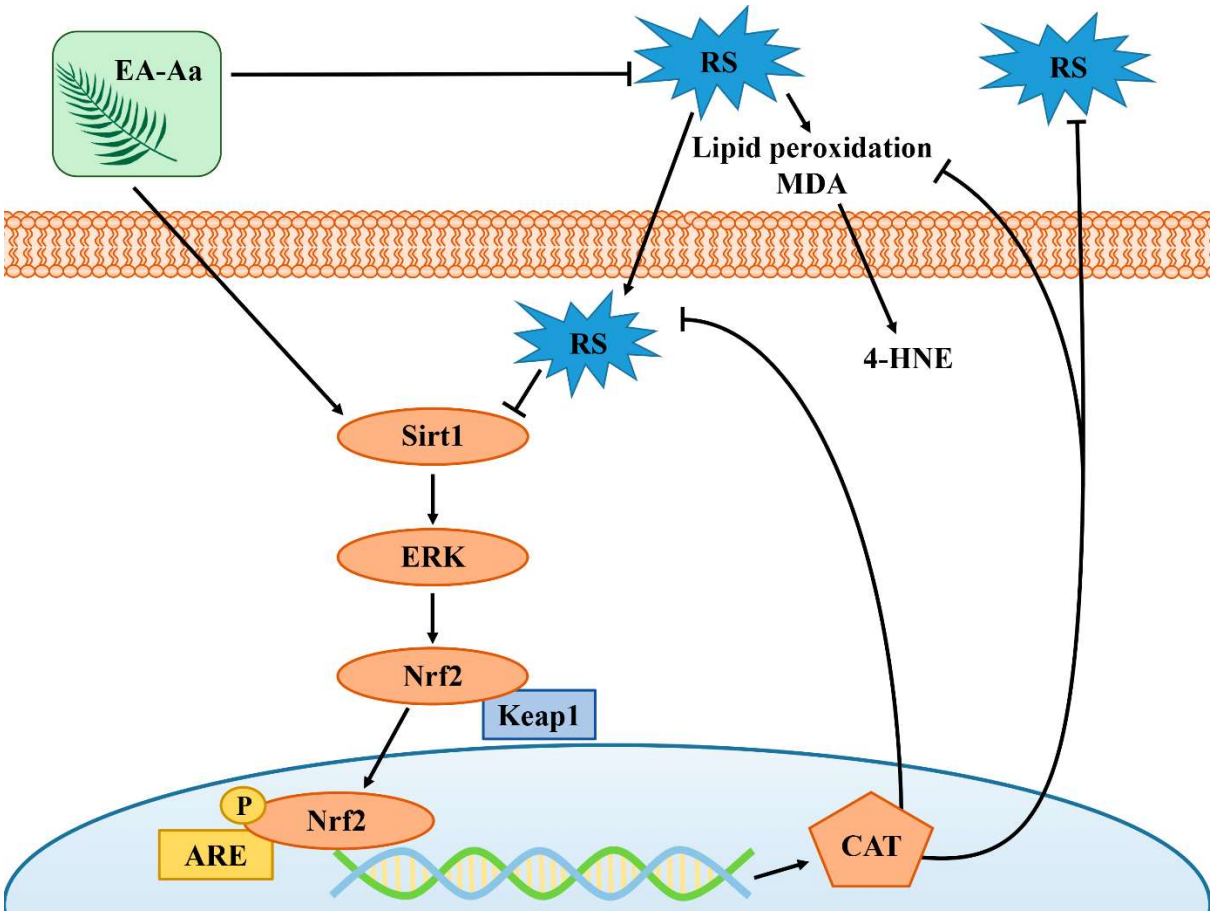


Figure 13. Antioxidant mechanism of action of NRF2/SIRT1 association activated by a plant extract from the Brazilian Cerrado. EA-Aa acts through the cascade of proteins responsible for the activation and transcription of antioxidant protection mechanisms. EA-Aa - Aqueous extract of *A. aculeata* leaves; SIRT1 - sirtuin; ERK - extracellular signal-regulated kinase; NRF2 - nuclear factor erythroid 2–related factor 2; Keap1 – kelch-like ECH-associated protein 1; ARE – antioxidante response elements; CAT - catalase; RS – reactive species; MDA – malondialdehyde; 4 – HNE - 4-hydroxy-2-nonenal (MONTEIRO-ALFREDO, 2020).

The alternative pathway activators of NRF2 phosphorylate it in serine and threonine residues and promote Keap1 dissociation and translocation to the nucleus, as is the case of protein kinases, such as PKC, JNK, ERK, and PI3K (KASPAR; NITURE; JAISWAL, 2009). There are reports that the enzyme glycogen synthase kinase-3β also regulates NRF2, but negatively inhibiting its action, but little is known about this until now (CULBRETH; ASCHNER, 2018). Finally, NRF2 itself can also promote self-regulation, as a regulatory loop, for maintaining the amount of NRF2 and Keap1 in the cell, in this way NRF2 itself controls the degradation, expression and induction of the gene of Keap1 (LEE et al., 2007).

NRF2 is also a regulator of proinflammatory cytokine transcription genes, where it acts as an inhibitor of inflammation. Its function is also involved in regulating genes of lipid metabolism, induction of autophagy and cellular apoptosis (autophagy-related protein 5-ATG5)

(LAZARO et al., 2018), it is able to improve hypothalamic insulin and leptin resistance when under oxidative stress conditions (YAGISHITA et al., 2017). In the case of DM2, NRF2 can also be a protective target against uncontrolled inflammation, oxidative stress, and micro and macrovascular complications, in addition to promoting atheroprotection (LAZARO et al., 2018). It also is involved in the regulation of lipogenesis genes (WANG et al., 2020) and protects against weight gain and promotes the formation of small adipocytes. Its deficiency can lead to reduced expression of peroxisome proliferator-activated receptor γ (PPAR γ), CCAAT enhancer-binding protein α (CEBPα), showing that, in addition to acting in detoxification pathways of phase II, NRF2 also plays an important role in the differentiation of preadipocytes and the expression of adipogenic transcription factors, in specific PPAR γ promoter sites (PI et al., 2010).

In a series of studies carried out with knockout animals, the importance of NRF2 in several metabolic and immunological pathways of the organism was proven. When NRF2 was suppressed, animals developed ocular pathology (ZHAO et al., 2011), oxidative injury, inflammation (BOYANAPALLI et al., 2014) and lower expression of antioxidant enzymes (KHOR et al., 2008). NRF2 deficiency was also related with greater chances of developing Alzheimer's disease, presenting a phenotype with higher oxidative and pro-inflammatory markers (REN et al., 2020), the development of systemic autoimmune disease (LI; STEIN; JOHNSON, 2004), increase of erythrocyte sensitization and the development of hemolytic anemia (LEE et al., 2004), and the predisposition to neutrophilic inflammation with the development of pulmonary emphysema (IIZUKA et al., 2005), and asthma (RANGASAMY et al., 2005). In addition, NRF2 deficiency leads to a greater propensity to develop malignant lesions and breast carcinomas, lung adenomas, with NF-κB activation (BECKS et al., 2010), greater susceptibility to colorectal carcinogenesis (KHOR et al., 2008), and liver and skin cancer (BEYER et al., 2008). Altogether, these studies prove that NRF2 is much more than an important target in the maintenance of redox balance, but rather a crucial systemic regulator of the function of various organs and mechanisms.

About the clinical studies, there are pharmacological agents capable of modulating such transcriptional factors, acting as therapeutic alternatives for the treatment of oxidative stress-related diseases. Examples of NRF2-associated pathway modulators are resveratrol, curcumin, melatonin (OOI et al., 2018), gallic acid (FENG et al., 2018) and ferulic acid (HOU; ZHANG; YANG, 2019). In preclinical studies, the pharmacological activators of NRF2 are isopropyl sulfur cyanogen compounds (sulforaphane), 1,2-mercapto-3-sulfur ketone derivatives

(oltipraz), selenium-containing drugs (ebselen) (LAZARO et al., 2018) and the phenolic compounds (HAN et al., 2008).

The study of phytochemical compounds, especially phenolic compounds, which can help to activate transcription factors such as NRF2 is an important area of research. They seek to identify the role between mechanisms of action and phytochemicals (BOYANAPALLI et al., 2014), in order to develop new alternatives. In this perspective, we bring studies that associate the therapeutic activity of NRF2 and other pathways with plants mainly constituted by this class of secondary metabolites. One example of a pathway associated with NRF2 is the nuclear factor kappa B (NF-κB), NRF2/NF-κB, activated by phenolic compounds such as the case of the plant *Molineria latifolia*. It has the effect of reestablishing the redox imbalance caused by DM2, through the tissue-specific modulation of the antioxidant response (OOI et al., 2018). Curcumin is another example of a phenolic compound that mediates the pathway involving the electrophile responsive element (EpRE), NRF2/EpRE and has been shown to have a hepatoprotective effect (FAROMBI et al., 2008). This pathway seems to be activated by some metabolites of quercetin, which causes an increase in detoxifying enzymes (LEE-HILZ et al., 2006). As well, *Nymphae nouchali*, a plant rich in phenolic compounds, is capable of acting as antioxidant, more specifically in the cellular ROS scavenging activity through the NRF2 pathway associated with ERK/p38 pathway (BAJPAI et al., 2018). The NRF2/SIRT1 association has reports of beneficial effects on hepatoprotection (SAYED et al., 2020), and there are reports about a Cerrado palm, *Acrocomia aculeata*, which confirmed this beneficial association regarding the antioxidant potential of this pathway in several experimental models (MONTEIRO-ALFREDO et al., 2020a). Moreover, in the context of Cerrado, we summarized the available data about its plants and the published information regarding NRF2 activation by phenolic compounds, but information is scarce and recent, further justifying the importance of developing research in this area (Table 3). The literature search was also performed in PubMed and Science Direct (until September 2021), with the search terms ‘phenolic compounds’, ‘Cerrado plant’, and ‘NRF2’.

Table 3: Medicinal plants from the Brazilian Cerrado that have their therapeutic properties associated with NRF2 pathway.

Cerrado medicinal plant	Biological properties	Reference
Acrocomia aculeata	*In vitro* and *in vivo* antioxidant capacity	(MONTEIRO-ALFREDO et al., 2020b)
Bactris setosa	Anti-aging and antioxidant activity	(DA CUNHA; ARRUDA, 2017)
Bactris setosa	Antioxidant activity	(FUSTINONI-REIS et al., 2016)
Campomanesia adamantium	Antioxidant and hepatoprotective capacity	(DE OLIVEIRA FERNANDES et al., 2015)
Caryocar coriaceum	Antileishmanial and antioxidant activity	(TOMIOTTO-PELLISSIER et al., 2018)
Tabebuia rosea	Antioxidant activity	(GARZÓN-CASTAÑO et al., 2020)

2.4.6 *Arecaceae*

Palm trees represent the greatest symbol of tropical forests since they mostly exist only in the tropics. In Brazil, there are 119 species belonging to 39 genera, found in different environments due to their high adaptive capacity. Their fruits are widely used, pulp for human consumption, the endocarp and epicarp as a source of biomass (DONATTI, C, 2004) and leaves are used for animal nutrition (CICONINI et al., 2013). The group of palm trees belonging to the *Arecaceae* family is one of the largest and has many species with important recognized economic value (DRANSFIELD, J; MANOKARAN, N, 1993; JONES, L, 1988). It has about 440 genera and 3,000 species of palm trees distributed worldwide. In South America, it occurs from the coast of Brazil to Argentina and Paraguay, where there are 36 genera, and 195 species (GIULIETTI et al., 2005).

Some studies show important biological activities of plants of the same family, such as antioxidants of *Copernicia prunifera* (Mill.) HE Moore, known as carnauba palm (RUFINO et al., 2010) and anti-inflammatory for *Syagrus oleracea* (Mart.) Becc., known as *catolé* (SARAIVA et al., 2015). Also, the methanolic extract of *Cocos nucifera* (L) displays antioxidant, cytoprotective, and antidiabetic potential. Phytochemical analyzes revealed the presence of polyphenols, phenolic acids, resins, flavonoids, proteins, and amino acids

(RENJITH; CHIKKU; RAJAMOHAN, 2013). Besides, the aqueous extract of *Arecastrum romanzoffianum* fruits has anti-inflammatory activity due to the compound galactomannoglucan, which acts in the initial stage of inflammation, and inhibits the formation of edema similarly to the already standardized compounds, indomethacin and dexamethasone (DA SILVA; PARENTE, 2010). The leaves of *Butia capitata* Becc showed several antioxidant activities, with the methanolic extract being the most active. Polar and non-polar leaf extracts showed anti-inflammatory activity, with non-polar extracts being the most effective, and whose potential was attributed to the presence of flavonoids, α-tocopherols and sterols (AMMAR et al., 2014). Among the palm trees of the *Areacacea* family, in this study, we highlight the species *A. aculeata* (Jacq.) Lodd. Ex Mart., a plant native to the Brazilian Cerrado. Its main phytogeographic, phytochemical, botanical characteristics, and applications, are presented below.

2.4.6.1 ***Acrocomia aculeata***

A. aculeata Jacq. (Lodd) ex. Mart (Figure 14) is a palm tree distributed in South America, in several countries such as Mexico, southern Paraguay, and northern Argentina (COIMBRA; JORGE, 2012; LORENZI, 2006). In Brazil, its distribution includes the states of Mato Grosso do Sul, Pará, São Paulo, and Rio de Janeiro, among others, and especially occurs in the Cerrado and Pantanal (LORENZI, 2006). As shown in Figure 14, it is a palm tree that has a height between 10 and 15 m, popularly known as *macaúba* or *bocaíuva* (KOPPER, A et al., 2009).

Figure 14. *A. aculeata* Jacq. (Lodd) ex. Mart (MONTEIRO-ALFREDO, 2016).

Its fruits, as shown in Figure 15, have a yellow-orange color and its pulp is widely used in cooking and can be consumed fresh or used in the production of sweets such as ice cream, cookies and pies (BORA; MOREIRA, 2003). In the Pantanal, the fruit is also prepared as flour and used in cooking (POETSCH, J; HAUPENTHAL, D, 2019). The oils extracted from the pulp and almonds of *A. aculeata* have great industrial and economic importance. The pulp oil has an intense orange color, with a high presence of oleic acid (MARIANO, R et al., 2010), β-carotene (SANJINEZ-ARGANDOÑA; CHUBA, 2011) and α-tocopherol (COIMBRA; JORGE, 2012). The almond, on the other hand, has a large amount of light-colored, high-quality oil, rich in lauric acid (BELTRÃO; OLIVEIRA, 2007) and oleic acid (AMARAL et al., 2011; HIANE et al., 2005).

Figure 15. *A. aculeata* fruit, peel, pulp and almond (MONTEIRO-ALFREDO, 2016).

The production capacity of palm oil is 10-fold greater than soybean oil per hectare (ROSCOE; RICHETTI; MARANHO, 2007). It is an important source of production of biokerosene (MANFIO et al., 2011) and biodiesel, with a production potential that reaches 4,000 tons of pulp oil.ha^{-1} (BASIRON, Y, 2005). Both pulp and almond have applications in the cosmetics industry, as well as in the pharmaceutical and food industry.

The leaves of *A. aculeata* (Figure 16) are used in animal nutrition due to the absence of toxic compounds (AMARAL, F et al., 2011; HIANE et al., 2005). The use of this palm has become widespread due to its important and diverse therapeutic effects, for example, in the treatment of respiratory diseases, or acting as laxative, analgesic (LORENZI, 2006) and anti-inflammatory (LESCANO et al., 2015). Beyond the reduction of glycemia and serum cholesterol levels (SILVA, 2012). Other properties of the plant were also reported, namely, in concerns to the prevention of bone problems, osteoarthritis, arthritis, and muscle pain, such as soothing (SARAIVA et al., 2015) and in the treatment of hypovitaminosis A (RAMOS et al., 2007). Therefore, about the *A. aculeata* leaves, a single report was recently published, as mentioned in previous topics, where the low toxicity of the leaves and the relevant antioxidant potential have been proven (MONTEIRO-ALFREDO et al., 2020a).

Figure 16. *A. aculeata* exsiccate deposited in the herbarium (DDMS-UFGD) of the Federal University of Grande Dourados, Dourados (MS), Brazil, registration number - 5103 (MONTEIRO-ALFREDO, 2016).

3 OBJECTIVES

3.1 General

Regarding the aqueous extract of *A. aculeata* (Jacq.) Lodd. ex Mart leaves (EA-Aa), the general aim was to evaluate its effect in relation to the:

3.1.1. Metabolic profile and protection against diabetes complications related to oxidative stress

3.1.2. Anticancer therapy and prevention of cardiotoxicity of doxorubicin chemotherapy

3.2 Specifics

The specific objectives about the pharmacological effect of EA-Aa were to evaluate its effect:

3.2.1. Target 1

- Metabolic profile of normal and diabetic rats;
- Oxidative stress in tissues involved in metabolic activity and targets of diabetic complications (adipose tissue, liver, heart, and kidney);
- Diabetic endothelial dysfunction and vascular oxidative stress *in vitro* and *in vivo*;
- Molecular mechanisms involved in the protection against oxidative stress-related complications.

3.2.2 Target 2:

-Normal and cancer cell viability;
-Molecular mechanisms of cell death and mitochondrial dysfunction;
-Oxidative hemolysis, lipid peroxidation, and ROS levels in normal cells;
-Acute toxicity *in vivo*;
- Dox-induced cardiotoxicity;
- Molecular mechanisms involved in cardioprotection.

4 REFERENCES

ABBOTT, C. A. et al. Prevalence and characteristics of painful diabetic neuropathy in a large community-based diabetic population in the U.K. **Diabetes Care**, v. 34, n. 10, p. 2220–2224, out. 2011.

Adriblastina® RD | Pfizer Brasil. Disponível em: <https://www.pfizer.com.br/bulas/adriblastina>. Acesso em: 6 out. 2021.

AGOSTINI-COSTA, T. DA S. Bioactive compounds and health benefits of some palm species traditionally used in Africa and the Americas – A review. **Journal of Ethnopharmacology**, v. 224, p. 202–229, 5 out. 2018.

AHMAD, M.; WOLBERG, A.; KAHWAJI, C. I. Biochemistry, Electron Transport Chain. In: **StatPearls**. Treasure Island (FL): StatPearls Publishing, 2021.

ALANAZI, A. et al. Antioxidant, antiapoptotic, and antifibrotic effects of the combination of liposomal resveratrol and carvedilol against doxorubicin-induced cardiomyopathy in rats. **Journal of Biochemical and Molecular Toxicology**, p. e22492, 15 mar. 2020.

ALARA, O. R.; ABDURAHMAN, N. H.; UKAEGBU, C. I. Extraction of phenolic compounds: A review. **Current Research in Food Science**, v. 4, p. 200–214, 1 jan. 2021.

AL-AUBAIDY, H. A.; JELINEK, H. F. Oxidative DNA damage and obesity in type 2 diabetes mellitus. **European Journal of Endocrinology**, v. 164, n. 6, p. 899–904, 1 jun. 2011.

ALGHAMDI, F.; ALSHUWEISHI, Y.; SALT, I. P. Regulation of nutrient uptake by AMP-activated protein kinase. **Cellular Signalling**, v. 76, p. 109807, 1 dez. 2020.

AMARAL, F et al. **Revista ENERGIA na agricultura**. Disponível em: <http://revistas.fca.unesp.br/index.php/energia/article/view/142>. Acesso em: 10 set. 2019.

American Cancer Society. Disponível em: <https://www.cancer.org/>. Acesso em: 6 out. 2021.

AMERICAN DIABETES ASSOCIATION. Nephropathy in Diabetes. **Diabetes Care**, v. 27, n. suppl 1, p. s79–s83, 1 jan. 2004.

AMMAR, N. M. et al. Phytochemical and biological studies of Butia capitata Becc. leaves cultivated in Egypt. **Asian Pacific Journal of Tropical Biomedicine**, v. 4, n. 6, p. 456–462, jun. 2014.

ANDRISIC, L. et al. Short overview on metabolomics approach to study pathophysiology of oxidative stress in cancer. **Redox Biology**, v. 14, p. 47–58, 1 abr. 2018.

ARAÚJO, J. **Química de Alimentos - Teoria e Prática - 7ª Edição**. Disponível em: <https://www.agrolivros.com.br/nutricao-e-tecnologia-de-alimentos/livro-quimica-de-alimentos-teoria-e-pratica--p>. Acesso em: 9 set. 2019.

ARRUDA, H. S. et al. Recent advances and possibilities for the use of plant phenolic compounds to manage ageing-related diseases. **Journal of Functional Foods**, v. 75, p. 104203, 1 dez. 2020.

ASANO, T. et al. Role of phosphatidylinositol 3-kinase activation on insulin action and its alteration in diabetic conditions. **Biological & Pharmaceutical Bulletin**, v. 30, n. 9, p. 1610–1616, set. 2007.

ATTWELL, D. et al. What is a pericyte? **Journal of Cerebral Blood Flow & Metabolism**, v. 36, n. 2, p. 451–455, fev. 2016.

AYALA, A.; MUÑOZ, M. F.; ARGÜELLES, S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. **Oxidative Medicine and Cellular Longevity**, v. 2014, p. 360438, 2014.

BAJPAI, V. K. et al. Antioxidant mechanism of polyphenol-rich Nymphaea nouchali leaf extract protecting DNA damage and attenuating oxidative stress-induced cell death via Nrf2-mediated heme-oxygenase-1 induction coupled with ERK/p38 signaling pathway. **Biomedicine & Pharmacotherapy**, v. 103, p. 1397–1407, 1 jul. 2018.

BAO, W. et al. Plasma Heme Oxygenase-1 Concentration Is Elevated in Individuals with Type 2 Diabetes Mellitus. **PLoS ONE**, v. 5, n. 8, p. e12371, 25 ago. 2010.

BARBOSA, K. B. F. et al. Estresse oxidativo: conceito, implicações e fatores modulatórios. **Revista de Nutrição**, v. 23, n. 4, p. 629–643, ago. 2010.

BARENHOLZ, Y. (CHEZY). Doxil® — The first FDA-approved nano-drug: Lessons learned. **Journal of Controlled Release**, Past, current and future applications of liposomes - Grand Challenges and Opportunities in Nanomedicine. v. 160, n. 2, p. 117–134, 10 jun. 2012.

BARG, S.; RORSMAN, P. Insulin Secretion. **The Journal of General Physiology**, v. 124, n. 6, p. 623–625, dez. 2004.

BASIRON, Y. **In: Bailey's Industrial Oil and Fat Products, Sixth Edition**. Disponível em: <https://www.feedipedia.org/node/15399>. Acesso em: 10 set. 2019.

BECKS, L. et al. Aggressive mammary carcinoma progression in Nrf2 knockout mice treated with 7,12-dimethylbenz[a]anthracene. **BMC Cancer**, v. 10, n. 1, p. 540, 8 out. 2010.

BELTRÃO, N.; OLIVEIRA, M. **Oleaginosas potenciais do Nordeste para a produção de biodiesel. - Portal Embrapa**. Disponível em: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/277417/oleaginosas-potenciais-do-nordeste-para-a-producao-de-biodiesel>. Acesso em: 9 set. 2019.

BENJANUWATTRA, J. et al. Doxorubicin and its proarrhythmic effects: A comprehensive review of the evidence from experimental and clinical studies. **Pharmacological Research**, v. 151, p. 104542, 1 jan. 2020.

BERTHIAUME, J. M.; WALLACE, K. B. Adriamycin-induced oxidative mitochondrial cardiotoxicity. **Cell Biology and Toxicology**, v. 23, n. 1, p. 15–25, jan. 2007.

BEYER, T. A. et al. Impaired liver regeneration in Nrf2 knockout mice: role of ROS-mediated insulin/IGF-1 resistance. **The EMBO Journal**, v. 27, n. 1, p. 212–223, 9 jan. 2008.

BIANCHI, M. DE L. P.; ANTUNES, L. M. G. Radicais livres e os principais antioxidantes da dieta. **Revista de Nutrição**, v. 12, n. 2, p. 123–130, ago. 1999.

BOEING, J. S. et al. Chemical characterization and protective effect of the Bactris setosa Mart. fruit against oxidative/nitrosative stress. **Food Chemistry**, v. 220, p. 427–437, 1 abr. 2017.

BONILHA, V. L. Oxidative Stress Regulation and DJ-1 Function in the Retinal Pigment Epithelium: Implications for AMD. **Advances in Experimental Medicine and Biology**, v. 1074, p. 3–9, 2018.

BORA, P. S.; MOREIRA, R. V. R. Catolé palm (Syagrus oleracea Mart) fruits: fatty and amino acids composition. **Grasas y Aceites**, v. 54, n. 2, p. 145–150, 30 jun. 2003.

BOYANAPALLI, S. S. S. et al. Nrf2 Knockout Attenuates the Anti-Inflammatory Effects of Phenethyl Isothiocyanate and Curcumin. **Chemical Research in Toxicology**, v. 27, n. 12, p. 2036–2043, 15 dez. 2014.

BREWER, P. D. et al. Insulin-regulated Glut4 Translocation. **The Journal of Biological Chemistry**, v. 289, n. 25, p. 17280–17298, 20 jun. 2014.

BROWNLEE, M. Biochemistry and molecular cell biology of diabetic complications. **Nature**, v. 414, n. 6865, p. 813–820, 13 dez. 2001.

BROWNLEE, M. The Pathobiology of Diabetic Complications: A Unifying Mechanism. **Diabetes**, v. 54, n. 6, p. 1615–1625, 1 jun. 2005.

BUSE, M. G. Hexosamines, insulin resistance and the complications of diabetes: current status. **American journal of physiology. Endocrinology and metabolism**, v. 290, n. 1, p. E1–E8, jan. 2006.

BUTOWSKA, K. et al. Cytotoxicity of doxorubicin conjugated with C60 fullerene. Structural and in vitro studies. **Structural Chemistry**, v. 30, n. 6, p. 2327–2338, 1 dez. 2019.

CADE, W. T. Diabetes-Related Microvascular and Macrovascular Diseases in the Physical Therapy Setting. **Physical Therapy**, v. 88, n. 11, p. 1322–1335, nov. 2008.

CADENAS, E.; SIES, H. Oxidative stress: Excited oxygen species and enzyme activity. **Advances in Enzyme Regulation**, v. 23, p. 217–237, 1 jan. 1985.

CAI XINYONG et al. The role of toll-like receptors in myocardial toxicity induced by doxorubicin. 2020.

CAMBOIM ROCKETT, F. et al. Phenolic compounds and antioxidant activity in vitro and in vivo of Butia and Opuntia fruits. **Food Research International**, v. 137, p. 109740, 1 nov. 2020.

CÂNDIDO, T. L. N.; SILVA, M. R.; AGOSTINI-COSTA, T. S. Bioactive compounds and antioxidant capacity of buriti (Mauritia flexuosa L.f.) from the Cerrado and Amazon biomes. **Food Chemistry**, v. 177, p. 313–319, 15 jun. 2015.

CANO, M. et al. Cigarette smoking, oxidative stress, the anti-oxidant response through Nrf2 signaling, and Age-related Macular Degeneration. **Vision Research**, Mechanisms in Macular Degeneration. v. 50, n. 7, p. 652–664, 31 mar. 2010.

CAPPETTA, D. et al. Oxidative Stress and Cellular Response to Doxorubicin: A Common Factor in the Complex Milieu of Anthracycline Cardiotoxicity. **Oxidative Medicine and Cellular Longevity**, v. 2017, 2017.

CAROCHO, M.; FERREIRA, I. C. F. R. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. **Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association**, v. 51, p. 15–25, jan. 2013.

CARVALHO, A. P. A. DE; CONTE-JUNIOR, C. A. Health benefits of phytochemicals from Brazilian native foods and plants: Antioxidant, antimicrobial, anti-cancer, and risk factors of metabolic/endocrine disorders control. **Trends in Food Science & Technology**, v. 111, p. 534–548, 1 maio 2021.

CASTRO, D. T. H. et al. Ethanolic Extract of Senna velutina Roots: Chemical Composition, In Vitro and In Vivo Antitumor Effects, and B16F10-Nex2 Melanoma Cell Death Mechanisms. **Oxidative Medicine and Cellular Longevity**, v. 2019, p. e5719483, 12 jun. 2019.

CÉSAR, A. DA S. et al. The prospects of using Acrocomia aculeata (macaúba) a non-edible biodiesel feedstock in Brazil. **Renewable and Sustainable Energy Reviews**, v. 49, p. 1213–1220, 1 set. 2015.

CHAN, W.-K. et al. Nitric oxide mediated endothelium-dependent relaxation induced by glibenclamide in rat isolated aorta. **Cardiovascular Research**, v. 46, n. 1, p. 180–187, 1 abr. 2000.

CHANDRAKUMAR, L. et al. Diabetes-Related Induction of the Heme Oxygenase System and Enhanced Colocalization of Heme Oxygenase 1 and 2 with Neuronal Nitric Oxide Synthase in Myenteric Neurons of Different Intestinal Segments.

Oxidative Medicine and Cellular Longevity, v. 2017, p. e1890512, 10 set. 2017.

CHAWLA, A.; CHAWLA, R.; JAGGI, S. Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum? **Indian Journal of Endocrinology and Metabolism**, v. 20, n. 4, p. 546–551, ago. 2016.

CHEN, Q. et al. Review: The role of NADP-malic enzyme in plants under stress. **Plant Science**, v. 281, p. 206–212, 1 abr. 2019.

CHEN, Q. M.; MALTAGLIATI, A. J. Nrf2 at the heart of oxidative stress and cardiac protection. **Physiological Genomics**, v. 50, n. 2, p. 77–97, 1 fev. 2018.

CHEN, Z. et al. Identification of phenolic compounds in Australian grown dragon fruits by LC-ESI-QTOF-MS/MS and determination of their antioxidant potential. **Arabian Journal of Chemistry**, v. 14, n. 6, p. 103151, 1 jun. 2021.

CHEUNG, Y.-M. M.; MCDONNELL, M.; HAMNVIK, O.-P. R. A targeted approach to phosphoinositide-3-kinase/Akt/mammalian target of rapamycin-induced hyperglycemia. **Current Problems in Cancer**, p. 100776, 24 jul. 2021.

CHRONIC KIDNEY DISEASE PROGNOSIS CONSORTIUM et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. **Lancet (London, England)**, v. 375, n. 9731, p. 2073–2081, 12 jun. 2010.

CICONINI, G. et al. Biometry and oil contents of Acrocomia aculeata fruits from the Cerrados and Pantanal biomes in Mato Grosso do Sul, Brazil. **Industrial Crops and Products**, v. 45, p. 208–214, 1 fev. 2013.

COIMBRA, M. C.; JORGE, N. Fatty acids and bioactive compounds of the pulps and kernels of Brazilian palm species, guariroba (Syagrus oleraces), jerivá (Syagrus romanzoffiana) and macaúba (Acrocomia aculeata). **Journal of the Science of Food and Agriculture**, v. 92, n. 3, p. 679–684, 2012.

CONKLIN, K. A. Chemotherapy-Associated Oxidative Stress: Impact on Chemotherapeutic Effectiveness. **Integrative Cancer Therapies**, v. 3, n. 4, p. 294–300, 1 dez. 2004.

COSTA, J. M. C.; OLIVEIRA, D. M.; COSTA, L. E. C. Macauba Palm—Acrocomia aculeata. In: RODRIGUES, S.; DE OLIVEIRA SILVA, E.; DE BRITO, E. S. (Eds.). . **Exotic Fruits**. [s.l.] Academic Press, 2018. p. 297–304.

CULBRETH, M.; ASCHNER, M. GSK-3β, a double-edged sword in Nrf2 regulation: Implications for neurological dysfunction and disease. **F1000Research**, v. 7, p. 1043, 10 jul. 2018.

DA CUNHA, M. D. S. B.; ARRUDA, S. F. Tucum-do-Cerrado (Bactris setosa Mart.) May Promote Anti-Aging Effect by Upregulating SIRT1-Nrf2 Pathway and Attenuating Oxidative Stress and Inflammation. **Nutrients**, v. 9, n. 11, p. 1243, nov. 2017.

DA SILVA, B. P.; PARENTE, J. P. Chemical properties and antiinflammatory activity of a galactomannoglucan from Arecastrum romanzoffianum. **Phytochemistry Letters**, v. 3, n. 3, p. 109–112, 20 set. 2010.

DAI, J.; MUMPER, R. J. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. **Molecules (Basel, Switzerland)**, v. 15, n. 10, p. 7313–7352, 21 out. 2010.

DARIO, M. F. et al. Synergistic photoprotective activity of nanocarrier containing oil of Acrocomia aculeata (Jacq.) Lodd. Ex. Martius—Arecaceae. **Industrial Crops and Products**, v. 112, p. 305–312, 1 fev. 2018.

DAVIES, M. et al. Effect of Oral Semaglutide Compared With Placebo and Subcutaneous Semaglutide on Glycemic Control in Patients With Type 2 Diabetes: A Randomized Clinical Trial. **JAMA**, v. 318, n. 15, p. 1460–1470, 17 out. 2017.

DE ALMEIDA, T. S. et al. Phenolic compounds of Triplaris gardneriana can protect cells against oxidative stress and restore oxidative balance. **Biomedicine & Pharmacotherapy**, v. 93, p. 1261–1268, 1 set. 2017.

DE BRITTO POLICARPI, P. et al. Nutritional potential, chemical profile and antioxidant activity of Chichá (Sterculia striata) nuts and its by-products. **Food Research International**, v. 106, p. 736–744, 1 abr. 2018.

DE CARVALHO, J. T. G. et al. The immunoregulatory function of polyphenols: implications in cancer immunity. **The Journal of Nutritional Biochemistry**, v. 85, p. 108428, 1 nov. 2020.
DE LIMA, J. P. et al. The antioxidative potential and volatile constituents of mangaba fruit over the storage period. **Scientia Horticulturae**, v. 194, p. 1–6, 14 out. 2015.

DE LUCA, V. et al. Mining the biodiversity of plants: a revolution in the making. **Science (New York, N.Y.)**, v. 336, n. 6089, p. 1658–1661, 29 jun. 2012.

DE OLIVEIRA FERNANDES, T. et al. Campomanesia adamantium (Myrtaceae) fruits protect HEPG2 cells against carbon tetrachloride-induced toxicity. **Toxicology Reports**, v. 2, p. 184–193, 1 jan. 2015.

DE SOUZA, F. G. et al. Brazilian fruits of Arecaceae family: An overview of some representatives with promising food, therapeutic and industrial applications. **Food Research International**, v. 138, p. 109690, 1 dez. 2020.

DE SOUZA, F. G. et al. Evaluation of antioxidant capacity, fatty acid profile, and bioactive compounds from buritirana (Mauritiella armata Mart.) oil: A little-explored native Brazilian fruit. **Food Research International**, v. 142, p. 110260, 1 abr. 2021.

DEL RÍO, J. C. et al. Chemical composition and thermal behavior of the pulp and kernel oils from macauba palm (Acrocomia aculeata) fruit. **Industrial Crops and Products**, v. 84, p. 294–304, 1 jun. 2016.

DONADO-PESTANA, C. M.; BELCHIOR, T.; GENOVESE, M. I. Phenolic compounds from cagaita (Eugenia dysenterica DC.) fruit prevent body weight and fat mass gain induced by a high-fat, high-sucrose diet. **Food Research International**, FOOD BIOACTIVE COMPOUNDS: QUALITY CONTROL AND BIOACTIVITY. v. 77, p. 177–185, 1 nov. 2015.

DONATTI, C. **Conseqüências da defaunação na dispersão e predação de sementes e no recrutamento...** Disponível em: <http://www.teses.usp.br/teses/disponiveis/91/91131/tde-20062005-170253/pt-br.php>. Acesso em: 9 set. 2019.

DONG, Q. Y. et al. Urinary 8-hydroxydeoxyguanosine levels in diabetic retinopathy patients. **European Journal of Ophthalmology**, v. 18, n. 1, p. 94–98, fev. 2008.

DOS REIS, S. O. et al. Juçara (Euterpe edulis Mart.) Supplementation Reduces Aberrant Crypt Foci and Increases SOD1 Expression in the Colorectal Mucosa of Carcinogenesis-Induced Rats. **Nutrition and Cancer**, v. 72, n. 4, p. 610–619, 2020.

DOS SANTOS DA ROCHA, P. et al. Microbiological quality, chemical profile as well as antioxidant and antidiabetic activities of Schinus terebinthifolius Raddi. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, v. 220, p. 36–46, 1 jun. 2019.

DOS SANTOS, J. M. et al. Guazuma ulmifolia Lam. Decreases Oxidative Stress in Blood Cells and Prevents Doxorubicin-Induced Cardiotoxicity. **Oxidative Medicine and Cellular Longevity**, v. 2018, p. e2935051, 28 jun. 2018a.

DOS SANTOS, K. P. et al. Biological activity of Hyptis Jacq. (Lamiaceae) is determined by the environment. **Industrial Crops and Products**, v. 112, p. 705–715, 1 fev. 2018b.

DRANSFIELD, J; MANOKARAN, N. **Plant resources of South-East Asia. No. 6**. Disponível em: <http://agris.fao.org/agris-search/search.do?recordID=XF2015005212>. Acesso em: 9 set. 2019.

DU, X.-L. et al. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. **Proceedings of the National Academy of Sciences of the United States of America**, v. 97, n. 22, p. 12222–12226, 24 out. 2000.

DZIEMIDOK, P.; DĄBROWSKI, M.; MAKARA-STUDZIŃSKA, M. Relationship between diabetic neuropathy and occurrence of depression among diabetic patients. **Psychiatria Polska**, v. 50, n. 2, p. 407–415, 2016.

EICKHOFF, H. et al. CIRURGIA METABÓLICA EM DOENTES COM DIABETES TIPO 2. FICÇÃO OU OPÇÃO TERAPÊUTICA? **Revista Portuguesa de Cirurgia**, v. 0, n. 36, p. 19–28, 30 mar. 2016.

ELDOR, R. et al. Glucose-reducing effect of the ORMD-0801 oral insulin preparation in patients with uncontrolled type 1 diabetes: a pilot study. **PloS One**, v. 8, n. 4, p. e59524, 2013.

ELSAYED, R. H. et al. Rumex dentatus L. phenolics ameliorate hyperglycemia by modulating hepatic key enzymes of carbohydrate metabolism, oxidative stress and PPARγ in diabetic rats. **Food and Chemical Toxicology**, v. 138, p. 111202, 1 abr. 2020.

ERLANK, H. et al. Polyphenols activate Nrf2 in astrocytes via H2O2, semiquinones, and quinones. **Free Radical Biology and Medicine**, v. 51, n. 12, p. 2319–2327, 15 dez. 2011.

ERSHOV, A. V.; BAZAN, N. G. Photoreceptor phagocytosis selectively activates PPARgamma expression in retinal pigment epithelial cells. **Journal of Neuroscience Research**, v. 60, n. 3, p. 328–337, 1 maio 2000.

EWER, M. S.; EWER, S. M. Cardiotoxicity of anticancer treatments: what the cardiologist needs to know. **Nature Reviews. Cardiology**, v. 7, n. 10, p. 564–575, out. 2010.

FAROMBI, E. O. et al. Curcumin attenuates dimethylnitrosamine-induced liver injury in rats through Nrf2-mediated induction of heme oxygenase-1. **Food and Chemical Toxicology**, Molecular and Physiological Effects of Bioactive Food Components. v. 46, n. 4, p. 1279–1287, 1 abr. 2008.

FASSEAS, M. K. et al. Antioxidant activity in meat treated with oregano and sage essential oils. **Food Chemistry**, v. 106, n. 3, p. 1188–1194, 1 fev. 2008.

FAVARETTO, V. F. et al. Differential responses of antioxidant enzymes in pioneer and late-successional tropical tree species grown under sun and shade conditions. **Environmental and Experimental Botany**, v. 70, n. 1, p. 20–28, 1 jan. 2011.

FENG, R.-B. et al. Gallic acid, a natural polyphenol, protects against tert-butyl hydroperoxide-induced hepatotoxicity by activating ERK-Nrf2-Keap1-mediated antioxidative response. **Food and Chemical Toxicology**, 3rd International Symposium on Phytochemicals in Medicine and Food (3-ISPMF). v. 119, p. 479–488, 1 set. 2018.

FERNANDES, G. W. et al. Deep into the mud: ecological and socio-economic impacts of the dam breach in Mariana, Brazil. **Natureza & Conservação**, v. 14, n. 2, p. 35–45, 1 jul. 2016.

FERNIE, A. R.; CARRARI, F.; SWEETLOVE, L. J. Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport. **Current Opinion in Plant Biology**, v. 7, n. 3, p. 254–261, 1 jun. 2004.

FIGUEIREDO DE SANTANA AQUINO, D. et al. Investigation of the antioxidant and hypoglycemiant properties of Alibertia edulis (L.C. Rich.) A.C. Rich. leaves. **Journal of Ethnopharmacology**, v. 253, p. 112648, 10 maio 2020.

FINKEL, T. Signal transduction by reactive oxygen species. **The Journal of Cell Biology**, v. 194, n. 1, p. 7–15, 11 jul. 2011.

FINKEL, T.; HOLBROOK, N. J. Oxidants, oxidative stress and the biology of ageing. **Nature**, v. 408, n. 6809, p. 239–247, 9 nov. 2000.

FONSECA, C. R.; VENTICINQUE, E. M. Biodiversity conservation gaps in Brazil: A role for systematic conservation planning. **Perspectives in Ecology and Conservation**, v. 16, n. 2, p. 61–67, 1 abr. 2018.

FORBES, J. M.; COOPER, M. E. Mechanisms of Diabetic Complications. **Physiological Reviews**, v. 93, n. 1, p. 137–188, 1 jan. 2013.

FOWLER, M. J. Microvascular and Macrovascular Complications of Diabetes. **Clinical Diabetes**, v. 26, n. 2, p. 77–82, 1 abr. 2008.

FRANCO, R. R. et al. Phytoscreening of Vochysiaceae species: Molecular identification by HPLC-ESI-MS/MS and evaluating of their antioxidant activity and inhibitory potential against human α-amylase and protein glycation. **Bioorganic Chemistry**, v. 91, p. 103122, 1 out. 2019.

FU, Y. et al. Facilitative glucose transporter gene expression in human lymphocytes, monocytes, and macrophages: a role for GLUT isoforms 1, 3, and 5 in the immune response

and foam cell formation. **Blood Cells, Molecules, and Diseases**, Stem Cell Plasticity. v. 32, n. 1, p. 182–190, 1 jan. 2004.

FU, Z.; GILBERT, E. R.; LIU, D. Regulation of Insulin Synthesis and Secretion and Pancreatic Beta-Cell Dysfunction in Diabetes. **Current diabetes reviews**, v. 9, n. 1, p. 25–53, 1 jan. 2013.

FUSTINONI-REIS, A. M. et al. Tucum-Do-Cerrado (Bactris setosa Mart.) Consumption Modulates Iron Homeostasis and Prevents Iron-Induced Oxidative Stress in the Rat Liver. **Nutrients**, v. 8, n. 2, p. 38, fev. 2016.

GALASSO, M. et al. Browsing the oldest antioxidant enzyme: catalase and its multiple regulation in cancer. **Free Radical Biology and Medicine**, v. 172, p. 264–272, 20 ago. 2021.

GALICIA-GARCIA, U. et al. Pathophysiology of Type 2 Diabetes Mellitus. **International Journal of Molecular Sciences**, v. 21, n. 17, p. 6275, 30 ago. 2020.

GALÚCIO, J. M. et al. In silico identification of natural products with anticancer activity using a chemo-structural database of Brazilian biodiversity. **Computational Biology and Chemistry**, v. 83, p. 107102, 1 dez. 2019.

GARZÓN-CASTAÑO, S. C. et al. Activation of the Keap1-Nrf2 pathway by specioside and the n-butanol extract from the inner bark of Tabebuia rosea (Bertol) DC. **F1000Research**, v. 9, p. 1262, 2 dez. 2020.

GELMAN, L.; FEIGE, J. N.; DESVERGNE, B. Molecular basis of selective PPARγ modulation for the treatment of Type 2 diabetes. **Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids**, PPARs. v. 1771, n. 8, p. 1094–1107, 1 ago. 2007.

GIACCO, F.; BROWNLEE, M. Oxidative stress and diabetic complications. **Circulation research**, v. 107, n. 9, p. 1058–1070, 29 out. 2010.

GIORDANI, M. A. et al. Hydroethanolic extract of the inner stem bark of Cedrela odorata has low toxicity and reduces hyperglycemia induced by an overload of sucrose and glucose. **Journal of Ethnopharmacology**, v. 162, p. 352–361, 13 mar. 2015.

GIUGLIANO, D. et al. Simplification of complex insulin therapy: a story of dogma and therapeutic resignation. **Diabetes Research and Clinical Practice**, v. 178, p. 108958, 1 ago. 2021.

GIULIETTI, A. M. et al. Biodiversity and Conservation of Plants in Brazil. **Conservation Biology**, v. 19, n. 3, p. 632–639, 2005.

GOMES, L. et al. Agricultural Expansion in the Brazilian Cerrado: Increased Soil and Nutrient Losses and Decreased Agricultural Productivity. **Land**, v. 8, n. 1, p. 12, jan. 2019.

GRIVENNIKOVA, V. G.; KAREYEVA, A. V.; VINOGRADOV, A. D. What are the sources of hydrogen peroxide production by heart mitochondria? **Biochimica et Biophysica Acta (BBA) - Bioenergetics**, 16th European Bioenergetics Conference 2010. v. 1797, n. 6, p. 939–944, 1 jun. 2010.

GUVEN, C.; SEVGILER, Y.; TASKIN, E. **Mitochondrial Dysfunction Associated with Doxorubicin**. [s.l.] IntechOpen, 2018.

HALLIWELL, B.; GUTTERIDGE, J. M. The importance of free radicals and catalytic metal ions in human diseases. **Molecular Aspects of Medicine**, v. 8, n. 2, p. 89–193, 1985.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free radicals in biology and medicine**. [s.l.] Clarendon Press, 1989.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free Radicals in Biology and Medicine**. [s.l.] Oxford University Press, 2015.

HAM, J. et al. Synthetic phenolic antioxidant propyl gallate induces male infertility through disruption of calcium homeostasis and mitochondrial function. **Environmental Pollution**, v. 248, p. 845–856, 1 maio 2019.

HAN, X. et al. Naringenin-7-O-glucoside protects against doxorubicin-induced toxicity in H9c2 cardiomyocytes by induction of endogenous antioxidant enzymes. **Food and Chemical Toxicology**, v. 46, n. 9, p. 3140–3146, 1 set. 2008.

HANG, Y. et al. The MafA Transcription Factor Becomes Essential to Islet β-Cells Soon After Birth. **Diabetes**, v. 63, n. 6, p. 1994–2005, 1 jun. 2014.

HARMAN, D. Free Radical Theory of Aging: An Update. **Annals of the New York Academy of Sciences**, v. 1067, n. 1, p. 10–21, 2006.

HARTWIG, A.; SCHWERDTLE, T. Interactions by carcinogenic metal compounds with DNA repair processes: toxicological implications. **Toxicology Letters**, v. 127, n. 1, p. 47–54, 28 fev. 2002.

HIANE, P. A. et al. Bocaiúva, Acrocomia aculeata (Jacq.) Lodd., pulp and kernel oils: characterization and fatty acid composition. **Brazilian Journal of Food Technology**, v. 8, p. 256–259, 1 jan. 2005.

HOSSAIN, U. et al. An overview on the role of bioactive α-glucosidase inhibitors in ameliorating diabetic complications. **Food and Chemical Toxicology**, v. 145, p. 111738, 1 nov. 2020.

HOU, T.; ZHANG, L.; YANG, X. Ferulic acid, a natural polyphenol, protects against osteoporosis by activating SIRT1 and NF-κB in neonatal rats with glucocorticoid-induced osteoporosis. **Biomedicine & Pharmacotherapy**, v. 120, p. 109205, 1 dez. 2019.

HUANG, W.-Y.; CAI, Y.-Z.; ZHANG, Y. Natural Phenolic Compounds From Medicinal Herbs and Dietary Plants: Potential Use for Cancer Prevention. **Nutrition and Cancer**, v. 62, n. 1, p. 1–20, 31 dez. 2009.

IGHODARO, O. M. Molecular pathways associated with oxidative stress in diabetes mellitus. **Biomedicine & Pharmacotherapy**, v. 108, p. 656–662, 1 dez. 2018.

IIZUKA, T. et al. Nrf2-deficient mice are highly susceptible to cigarette smoke-induced emphysema. **Genes to Cells**, v. 10, n. 12, p. 1113–1125, 2005.

INCA - Instituto Nacional de Câncer. Disponível em: <https://www.inca.gov.br/>. Acesso em: 6 out. 2021.

International Diabetes Federation - Facts and Figures. Disponível em: <https://idf.org/>. Acesso em: 20 jul. 2021.

JABBEHDARI, S.; HANDA, J. T. Oxidative stress as a therapeutic target for the prevention and treatment of early age-related macular degeneration. **Survey of Ophthalmology**, v. 66, n. 3, p. 423–440, 1 maio 2021.

JAFARI, S.; SAEIDNIA, S.; ABDOLLAHI, M. Role of natural phenolic compounds in cancer chemoprevention via regulation of the cell cycle. **Current Pharmaceutical Biotechnology**, v. 15, n. 4, p. 409–421, 2014.

JEMIMA ROMOLA, C. V. et al. A comprehensive review of the selection of natural and synthetic antioxidants to enhance the oxidative stability of biodiesel. **Renewable and Sustainable Energy Reviews**, v. 145, p. 111109, 1 jul. 2021.

JIMENEZ-LUNA, C. et al. Novel Biomarkers to Distinguish between Type 3c and Type 2 Diabetes Mellitus by Untargeted Metabolomics. **Metabolites**, v. 10, n. 11, p. E423, 22 out. 2020.

JONES, L. **Palms in Australia**. Disponível em: <https://www.abebooks.com/book-search/title/palms-in-australia/author/jones-david/>. Acesso em: 9 set. 2019.

JUSTINO, A. B. et al. Annona muricata Linn. leaf as a source of antioxidant compounds with in vitro antidiabetic and inhibitory potential against α-amylase, α-glucosidase, lipase, non-enzymatic glycation and lipid peroxidation. **Biomedicine & Pharmacotherapy**, v. 100, p. 83–92, 1 abr. 2018.

KALRA, S. Sodium Glucose Co-Transporter-2 (SGLT2) Inhibitors: A Review of Their Basic and Clinical Pharmacology. **Diabetes Therapy**, v. 5, n. 2, p. 355–366, dez. 2014.

KANEHIRA, Y. et al. Tumor distribution and anti-tumor effect of doxorubicin following intrapulmonary administration to mice with metastatic lung tumor. **Journal of Drug Delivery Science and Technology**, v. 33, p. 143–148, 1 jun. 2016.

KANETO, H.; MATSUOKA, T. Involvement of Oxidative Stress in Suppression of Insulin Biosynthesis under Diabetic Conditions. **International Journal of Molecular Sciences**, v. 13, n. 10, p. 13680–13690, 22 out. 2012.

KANG, Q.; YANG, C. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. **Redox Biology**, v. 37, p. 101799, 1 out. 2020.

KARYLOWSKI, O. et al. GLUT4 is retained by an intracellular cycle of vesicle formation and fusion with endosomes. **Molecular Biology of the Cell**, v. 15, n. 2, p. 870–882, fev. 2004.

KASMI, S. et al. Characterization of phenolic compounds isolated from the Fraxinus angustifolia plant and several associated bioactivities. **Journal of Herbal Medicine**, v. 29, p. 100485, 1 out. 2021.

KASPAR, J. W.; NITURE, S. K.; JAISWAL, A. K. Nrf2:INrf2 (Keap1) signaling in oxidative stress. **Free Radical Biology and Medicine**, Special Issue on Redox Signalling. v. 47, n. 9, p. 1304–1309, 1 nov. 2009.

KAVAZIS, A. N. et al. Effects of doxorubicin on cardiac muscle subsarcolemmal and intermyofibrillar mitochondria. **Mitochondrion**, v. 34, p. 9–19, maio 2017.

KAWAHITO, S.; KITAHATA, H.; OSHITA, S. Problems associated with glucose toxicity: Role of hyperglycemia-induced oxidative stress. **World Journal of Gastroenterology : WJG**, v. 15, n. 33, p. 4137–4142, 7 set. 2009.

KHEDKAR, A. et al. A dose range finding study of novel oral insulin (IN-105) under fed conditions in type 2 diabetes mellitus subjects. **Diabetes, Obesity & Metabolism**, v. 12, n. 8, p. 659–664, ago. 2010.

KHOR, T. O. et al. Increased Susceptibility of Nrf2 Knockout Mice to Colitis-Associated Colorectal Cancer. **Cancer Prevention Research**, v. 1, n. 3, p. 187–191, 1 ago. 2008.

KIKUCHI, S. et al. Glycation—a sweet tempter for neuronal death. **Brain Research Reviews**, v. 41, n. 2, p. 306–323, 1 mar. 2003.

KOPPER, A et al. **UTILIZAÇÃO TECNOLÓGICA DA FARINHA DE BOCAIUVA NA ELABORAÇÃO DE BISCOITOS TIPO COOKIE**. Disponível em: <https://docplayer.com.br/46332229-Utilizacao-tecnologica-da-farinha-de-bocaiuva-na-elaboracao-de-biscoitos-tipo-cookie.html>. Acesso em: 10 set. 2019.

KOWLURU, R. A.; CHAN, P.-S. Oxidative stress and diabetic retinopathy. **Experimental Diabetes Research**, v. 2007, p. 43603, 2007.

KOYA, D.; KING, G. L. Protein kinase C activation and the development of diabetic complications. **Diabetes**, v. 47, n. 6, p. 859–866, jun. 1998.

KUMAR, S.; PANDEY, A. K. **Chemistry and Biological Activities of Flavonoids: An Overview**. Review Article. Disponível em: <https://www.hindawi.com/journals/tswj/2013/162750/>. Acesso em: 25 jan. 2021.

KUWABARA, W. M. T. et al. Obesity and Type 2 Diabetes mellitus induce lipopolysaccharide tolerance in rat neutrophils. **Scientific Reports**, v. 8, n. 1, p. 17534, 3 dez. 2018.

LAMMING, D. W. et al. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. **Science (New York, N.y.)**, v. 335, n. 6076, p. 1638–1643, 30 mar. 2012.

LAZARO, I. et al. Nrf2 Activation Provides Atheroprotection in Diabetic Mice Through Concerted Upregulation of Antioxidant, Anti-inflammatory, and Autophagy Mechanisms. **Frontiers in Pharmacology**, v. 9, p. 819, 2018.

LE BOT, M. A. et al. Different cytotoxicity and metabolism of doxorubicin, daunorubicin, epirubicin, esorubicin and idarubicin in cultured human and rat hepatocytes. **Biochemical Pharmacology**, v. 37, n. 20, p. 3877–3887, 15 out. 1988.

LEÃO, A. D. et al. Strategies to improve glibenclamide dissolution: A review using database tomography. **Journal of Drug Delivery Science and Technology**, v. 54, p. 101242, 1 dez. 2019.

LEDO, A. et al. Human milk enhances antioxidant defenses against hydroxyl radical aggression in preterm infants. **The American Journal of Clinical Nutrition**, v. 89, n. 1, p. 210–215, 1 jan. 2009.

LEE, J.-M. et al. Targeted disruption of Nrf2 causes regenerative immune-mediated hemolytic anemia. **Proceedings of the National Academy of Sciences**, v. 101, n. 26, p. 9751–9756, 29 jun. 2004.

LEE, O.-H. et al. An auto-regulatory loop between stress sensors INrf2 and Nrf2 controls their cellular abundance. **The Journal of Biological Chemistry**, v. 282, n. 50, p. 36412–36420, 14 dez. 2007.

LEE-HILZ, Y. Y. et al. Pro-oxidant activity of flavonoids induces EpRE-mediated gene expression. **Chemical Research in Toxicology**, v. 19, n. 11, p. 1499–1505, nov. 2006.

LEITE, N. R. et al. Baru Pulp (Dipteryx alata Vogel): Fruit from the Brazilian Savanna Protects against Oxidative Stress and Increases the Life Expectancy of Caenorhabditis elegans via SOD-3 and DAF-16. **Biomolecules**, v. 10, n. 8, p. E1106, 25 jul. 2020.

LEMOS, M. R. B. et al. The effect of roasting on the phenolic compounds and antioxidant potential of baru nuts [Dipteryx alata Vog.]. **Food Research International**, v. 48, n. 2, p. 592–597, 1 out. 2012.

LESCANO, C. H. et al. Diuretic and Anti-Inflammatory Activities of the Microencapsulated Acrocomia aculeata (Arecaceae) Oil on Wistar Rats. **Journal of Medicinal Food**, v. 18, n. 6, p. 656–662, jun. 2015.

LETO, D.; SALTIEL, A. R. Regulation of glucose transport by insulin: traffic control of GLUT4. **Nature Reviews. Molecular Cell Biology**, v. 13, n. 6, p. 383–396, 23 maio 2012.

LI, J.; STEIN, T. D.; JOHNSON, J. A. Genetic dissection of systemic autoimmune disease in Nrf2-deficient mice. **Physiological Genomics**, v. 18, n. 3, p. 261–272, 11 ago. 2004.

LIU, T. et al. NF-κB signaling in inflammation. **Signal Transduction and Targeted Therapy**, v. 2, n. 1, p. 1–9, 14 jul. 2017.

LOBO, V. et al. Free radicals, antioxidants and functional foods: Impact on human health. **Pharmacognosy Reviews**, v. 4, n. 8, p. 118–126, 2010.

LORENZI, G. M. A. C. Acrocomia aculeata (Jacq.) Lodd.ex Mart. - Arecaceae : bases para o extrativismo sustentável /. **oai:ufpr.br:222404**, 2006.

LORENZI, M. The Polyol Pathway as a Mechanism for Diabetic Retinopathy: Attractive, Elusive, and Resilient. **Experimental Diabetes Research**, v. 2007, p. 61038, 2007.

MA, Q. Role of nrf2 in oxidative stress and toxicity. **Annual Review of Pharmacology and Toxicology**, v. 53, p. 401–426, 2013.

MAAMOUN, H. et al. Crosstalk Between Oxidative Stress and Endoplasmic Reticulum (ER) Stress in Endothelial Dysfunction and Aberrant Angiogenesis Associated With Diabetes: A Focus on the Protective Roles of Heme Oxygenase (HO)-1. **Frontiers in Physiology**, v. 10, 2019.

MAES, M. et al. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 35, n. 3, p. 676–692, 29 abr. 2011.

MAJIMA, H. J. et al. Chasing great paths of Helmut Sies "Oxidative Stress". **Archives of Biochemistry and Biophysics**, Special Issue: Tribute Issue: Helmut Sies and Oxidative Stress: venit, vidit, vicit. v. 595, p. 54–60, 1 abr. 2016.

MALTA, L. G. et al. In vivo analysis of antigenotoxic and antimutagenic properties of two Brazilian Cerrado fruits and the identification of phenolic phytochemicals. **Food Research International**, v. 49, n. 1, p. 604–611, 1 nov. 2012.

MANFIO, C. E. et al. Repeatability in biometric characteristics of macaw palm fruit. **Ciência Rural**, v. 41, n. 1, p. 70–76, 2011.

MANQUIÁN-CERDA, K. et al. Interactive effects of aluminum and cadmium on phenolic compounds, antioxidant enzyme activity and oxidative stress in blueberry (Vaccinium corymbosum L.) plantlets cultivated in vitro. **Ecotoxicology and Environmental Safety**, v. 150, p. 320–326, 15 abr. 2018.

MARIANO, R et al. **Partition of free fatty acids in deacidification of macaúba (Acrocomia aculeata) pulp oil by liquid-liquid extraction using ethanol/water solution as solvent. - Portal Embrapa**. Disponível em: <https://www.embrapa.br/busca-de-publicacoes/-/publicacao/878182/partition-of-free-fatty-acids-in-deacidification-of-macauba-acrocomia-aculeata-pulp-oil-by-liquid-liquid-extraction-using-ethanolwater-solution-as-solvent>. Acesso em: 9 set. 2019.

MARINOVA, D.; RIBAROVA, F.; ATANASSOVA, M. TOTAL PHENOLICS AND TOTAL FLAVONOIDS IN BULGARIAN FRUITS AND VEGETABLES. p. 6, 2005.

MARÍN-PEÑALVER, J. J. et al. Update on the treatment of type 2 diabetes mellitus. **World Journal of Diabetes**, v. 7, n. 17, p. 354–395, 15 set. 2016.

MARSHALL, S.; BACOTE, V.; TRAXINGER, R. R. Discovery of a metabolic pathway mediating glucose-induced desensitization of the glucose transport system. Role of hexosamine biosynthesis in the induction of insulin resistance. **The Journal of Biological Chemistry**, v. 266, n. 8, p. 4706–4712, 15 mar. 1991.

MASTROTOTARO, L.; RODEN, M. Insulin resistance and insulin sensitizing agents. **Metabolism**, p. 154892, 23 set. 2021.

MAURYA, P. K. et al. The role of oxidative and nitrosative stress in accelerated aging and major depressive disorder. **Progress in Neuro-Psychopharmacology & Biological Psychiatry**, v. 65, p. 134–144, 4 fev. 2016.

MAYA-CANO, D. A.; ARANGO-VARELA, S.; SANTA-GONZALEZ, G. A. Phenolic compounds of blueberries (Vaccinium spp) as a protective strategy against skin cell damage induced by ROS: A review of antioxidant potential and antiproliferative capacity. **Heliyon**, v. 7, n. 2, p. e06297, 1 fev. 2021.

MCADAM, E.; BREM, R.; KARRAN, P. Oxidative stress-induced protein damage inhibits DNA repair and determines mutation risk and therapeutic efficacy. **Molecular Cancer Research**, 1 jan. 2016.

MEDINA, A. L. et al. Araçá (Psidium cattleianum Sabine) fruit extracts with antioxidant and antimicrobial activities and antiproliferative effect on human cancer cells. **Food Chemistry**, v. 128, n. 4, p. 916–922, 15 out. 2011.

MEDINA, R. A.; OWEN, G. I. Glucose transporters: expression, regulation and cancer. **Biological Research**, v. 35, n. 1, p. 9–26, 2002.

MEERWALDT, R. et al. The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. **Cardiovascular Diabetology**, v. 7, n. 1, p. 29, 7 out. 2008.

MEHDI, M. M.; SOLANKI, P.; SINGH, P. Oxidative stress, antioxidants, hormesis and calorie restriction: The current perspective in the biology of aging. **Archives of Gerontology and Geriatrics**, v. 95, p. 104413, 1 jul. 2021.

MELO, P. S. et al. Açaí seeds: An unexplored agro-industrial residue as a potential source of lipids, fibers, and antioxidant phenolic compounds. **Industrial Crops and Products**, v. 161, p. 113204, 1 mar. 2021.

Ministério da Saúde. Disponível em: <http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2005/int0005_11_12_2008.html>. Acesso em: 2 maio. 2021.

MIRSKY, I. A.; PERISUTTI, G. THE RELATIVE SPECIFICITY OF THE INSULINASE ACTIVITY OF RAT LIVER EXTRACTS. **Journal of Biological Chemistry**, v. 228, n. 1, p. 77–83, set. 1957.

MISHRA, M.; DURAISAMY, A. J.; KOWLURU, R. A. Sirt1: A Guardian of the Development of Diabetic Retinopathy. **Diabetes**, v. 67, n. 4, p. 745–754, 1 abr. 2018.

MMA, M. DO M. A. **O Bioma Cerrado**. Disponível em: <https://www.mma.gov.br/biomas/cerrado>. Acesso em: 29 abr. 2020.

MOBARAKI, M. et al. Molecular Mechanisms of Cardiotoxicity: A Review on Major Side-effect of Doxorubicin. **Indian Journal of Pharmaceutical Sciences**, v. 79, n. 3, p. 335–344, 31 jul. 2017.

MOGENSEN, C. E.; CHRISTENSEN, C. K.; VITTINGHUS, E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. **Diabetes**, v. 32 Suppl 2, p. 64–78, maio 1983.

MONDAL, M. et al. Protective effect of Bridelia tomentosa due to its phenolic acids and flavonoids against oxidative stress-mediated hepatic toxicity induced by carbofuran. **South African Journal of Botany**, v. 141, p. 447–456, 1 set. 2021.

MONTEIRO-ALFREDO, T. et al. **Acrocomia aculeata (Jacq.) Lodd. ex Mart. Leaves Increase SIRT1 Levels and Improve Stress Resistance**. Research Article. Disponível em: <https://www.hindawi.com/journals/omcl/2020/5238650/>. Acesso em: 20 mar. 2020a.

MONTEIRO-ALFREDO, T. et al. Acrocomia aculeata (Jacq.) Lodd. ex Mart. Leaves Increase SIRT1 Levels and Improve Stress Resistance. **Oxidative Medicine and Cellular Longevity**, v. 2020, p. e5238650, 10 mar. 2020b.

MONTEIRO-ALFREDO, T. et al. Hypoglycaemic and Antioxidant Properties of Acrocomia aculeata (Jacq.) Lodd Ex Mart. Extract Are Associated with Better Vascular Function of Type 2 Diabetic Rats. **Nutrients**, v. 13, n. 8, p. 2856, 20 ago. 2021.

MUECKLER, M.; THORENS, B. The SLC2 (GLUT) Family of Membrane Transporters. **Molecular aspects of medicine**, v. 34, n. 0, p. 121–138, 2013.

MUT-SALUD, N. et al. Antioxidant Intake and Antitumor Therapy: Toward Nutritional Recommendations for Optimal Results. **Oxidative Medicine and Cellular Longevity**, v. 2016, p. e6719534, 22 nov. 2015.

NAUMANN, H. D. et al. The role of condensed tannins in ruminant animal production: advances, limitations and future directions. **Revista Brasileira de Zootecnia**, v. 46, n. 12, p. 929–949, dez. 2017.

NEHA, K. et al. Medicinal prospects of antioxidants: A review. **European Journal of Medicinal Chemistry**, v. 178, p. 687–704, 15 set. 2019.

NERI-NUMA, I. A. et al. Evaluation of the antioxidant, antiproliferative and antimutagenic potential of araçá-boi fruit (Eugenia stipitata Mc Vaugh — Myrtaceae) of the Brazilian Amazon Forest. **Food Research International**, v. 50, n. 1, p. 70–76, 1 jan. 2013.

NISHIKAWA, T. et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. **Nature**, v. 404, n. 6779, p. 787–790, abr. 2000.

NOLFI-DONEGAN, D.; BRAGANZA, A.; SHIVA, S. Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement. **Redox Biology**, v. 37, p. 101674, 1 out. 2020.

OBROSOVA, I. G. Diabetic painful and insensate neuropathy: pathogenesis and potential treatments. **Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics**, v. 6, n. 4, p. 638–647, out. 2009.

OKELEJI, L. O. et al. Epidemiologic evidence linking oxidative stress and pulmonary function in healthy populations. **Chronic Diseases and Translational Medicine**, 29 jan. 2021.

OKPUZOR, J. et al. **In vitro Investigation of Antioxidant Phenolic Compounds in Extracts of Senna alata**. Disponível em: <https://scialert.net/abstract/?doi=rjphyto.2009.68.76>. Acesso em: 3 maio. 2021.

OLINTO, S. C. F. et al. Antiproliferative and Cytotoxic Effects of Schinus terebinthifolia Leaf Extract on Thyroid Follicular Cells. **Revista Brasileira de Farmacognosia**, v. 30, n. 5, p. 693–700, 1 out. 2020.

OLIVEIRA, V. B. et al. Native foods from Brazilian biodiversity as a source of bioactive compounds. **Food Research International**, v. 48, n. 1, p. 170–179, 1 ago. 2012.

OLIVEIRA-ALVES, S. C. et al. Identification of functional compounds in baru (Dipteryx alata Vog.) nuts: Nutritional value, volatile and phenolic composition, antioxidant activity and antiproliferative effect. **Food Research International**, v. 131, p. 109026, 1 maio 2020.

OOI, D. J. et al. Polyphenol-rich ethyl acetate fraction of Molineria latifolia rhizome restores oxidant-antioxidant balance by possible engagement of KEAP1-NRF2 and PKC/NF-κB signalling pathways. **Journal of Functional Foods**, v. 42, p. 111–121, 1 mar. 2018.

OUTUKI, P. M. et al. Development of arabic and xanthan gum microparticles loaded with an extract of Eschweilera nana Miers leaves with antioxidant capacity. **Colloids and Surfaces A: Physicochemical and Engineering Aspects**, v. 499, p. 103–112, 20 jun. 2016.

PALHARES, R. M. et al. Medicinal Plants Recommended by the World Health Organization: DNA Barcode Identification Associated with Chemical Analyses Guarantees Their Quality. **PLoS ONE**, v. 10, n. 5, 15 maio 2015.

PARK, S. et al. Vitamin C in Cancer: A Metabolomics Perspective. **Frontiers in Physiology**, v. 9, 19 jun. 2018.

PARVEZ, M. K. et al. New terpenic and phenolic compounds from Suaeda monoica reverse oxidative and apoptotic damages in human endothelial cells. **Saudi Pharmaceutical Journal**, 9 ago. 2021.

PENG, Y. et al. Absorption, metabolism, and bioactivity of vitexin: recent advances in understanding the efficacy of an important nutraceutical. **Critical Reviews in Food Science and Nutrition**, v. 61, n. 6, p. 1049–1064, 2021.

PEREIRA, A. P. A. et al. A comprehensive characterization of Solanum lycocarpum St. Hill and Solanum oocarpum Sendtn: Chemical composition and antioxidant properties. **Food Research International**, SLACA 2017: Food Science and its role in a changing World. v. 124, p. 61–69, 1 out. 2019.

PHANIENDRA, A.; JESTADI, D. B.; PERIYASAMY, L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. **Indian Journal of Clinical Biochemistry**, v. 30, n. 1, p. 11–26, jan. 2015.

PI, J. et al. Deficiency in the Nuclear Factor E2-related Factor-2 Transcription Factor Results in Impaired Adipogenesis and Protects against Diet-induced Obesity *. **Journal of Biological Chemistry**, v. 285, n. 12, p. 9292–9300, 19 mar. 2010.

PISOSCHI, A. M. et al. Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. **European Journal of Medicinal Chemistry**, v. 209, p. 112891, 1 jan. 2021.

PIVOVAROVA, O. et al. Insulin-degrading enzyme: new therapeutic target for diabetes and Alzheimer's disease? **Annals of Medicine**, v. 48, n. 8, p. 614–624, 16 nov. 2016.

PNPMF. **Programa Nacional de Plantas Medicinais e Fitoterápicos**. [s.l.] Ministério da Saúde (MS), 2009.

POETSCH, J; HAUPENTHAL, D. **Acrocomia aculeata – a sustainable oil crop**. Disponível em: <https://www.rural21.com/english/news/detail/article/acrocomia-aculeata-a-sustainable-oil-crop-0000420/>. Acesso em: 9 set. 2019.

PRADEEP, A. R. et al. 4-Hydroxy-2-nonenal, an oxidative stress marker in crevicular fluid and serum in type 2 diabetes with chronic periodontitis. **Contemporary Clinical Dentistry**, v. 4, n. 3, p. 281–285, jul. 2013.

PRADO, L. G. et al. Antioxidant, antiproliferative and healing properties of araticum (Annona crassiflora Mart.) peel and seed. **Food Research International**, v. 133, p. 109168, 1 jul. 2020.

PRESTI, G. et al. Bioactive compounds from extra virgin olive oils: Correlation between phenolic content and oxidative stress cell protection. **Biophysical Chemistry**, v. 230, p. 109–116, 1 nov. 2017.

PUGLIESE, G. et al. Mechanisms of glucose-enhanced extracellular matrix accumulation in rat glomerular mesangial cells. **Diabetes**, v. 43, n. 3, p. 478–490, mar. 1994.

QUARESMA, D. M. O. et al. Antioxidant compounds from Banisteriopsis argyrophylla leaves as α-amylase, α-glucosidase, lipase, and glycation inhibitors. **Bioorganic Chemistry**, v. 105, p. 104335, 1 dez. 2020.

RACHDI, L. et al. Disruption of Tsc2 in pancreatic beta cells induces beta cell mass expansion and improved glucose tolerance in a TORC1-dependent manner. **Proceedings of the National Academy of Sciences of the United States of America**, v. 105, n. 27, p. 9250–9255, 8 jul. 2008.

RADI, R. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. **Proceedings of the National Academy of Sciences**, v. 115, n. 23, p. 5839–5848, 5 jun. 2018.

RADI, R.; CASSINA, A.; HODARA, R. Nitric oxide and peroxynitrite interactions with mitochondria. **Biological Chemistry**, v. 383, n. 3–4, p. 401–409, abr. 2002.

RAMALHO, V. C.; JORGE, N. Antioxidants used in oils, fats and fatty foods. **Química Nova**, v. 29, n. 4, p. 755–760, jul. 2006.

RAMOS, M. I. L. et al. Bocaiuva (Acrocomia aculeata (Jacq.) Lodd) improved Vitamin A status in rats. **Journal of Agricultural and Food Chemistry**, v. 55, n. 8, p. 3186–3190, 18 abr. 2007.

RANGASAMY, T. et al. Disruption of Nrf2 enhances susceptibility to severe airway inflammation and asthma in mice. **Journal of Experimental Medicine**, v. 202, n. 1, p. 47–59, 4 jul. 2005.

RATTER, J. A.; RIBEIRO, J. F.; BRIDGEWATER, S. The Brazilian Cerrado Vegetation and Threats to its Biodiversity. **Annals of Botany**, v. 80, n. 3, p. 223–230, 1 set. 1997.

RECZEK, C. R. et al. A CRISPR screen identifies a pathway required for paraquat-induced cell death. **Nature Chemical Biology**, v. 13, n. 12, p. 1274–1279, dez. 2017.

REN, P. et al. Nrf2 Ablation Promotes Alzheimer's Disease-Like Pathology in APP/PS1 Transgenic Mice: The Role of Neuroinflammation and Oxidative Stress. **Oxidative Medicine and Cellular Longevity**, v. 2020, p. e3050971, 11 maio 2020.

RENJITH, R. S.; CHIKKU, A. M.; RAJAMOHAN, T. Cytoprotective, antihyperglycemic and phytochemical properties of Cocos nucifera (L.) inflorescence. **Asian Pacific Journal of Tropical Medicine**, v. 6, n. 10, p. 804–810, out. 2013.

RIBEIRO NETO, J. A. et al. Using the plants of Brazilian Cerrado for wound healing: From traditional use to scientific approach. **Journal of Ethnopharmacology**, v. 260, p. 112547, 5 out. 2020.

RIZVI, S. I.; MAURYA, P. K. Markers of oxidative stress in erythrocytes during aging in humans. **Annals of the New York Academy of Sciences**, v. 1100, p. 373–382, abr. 2007.

RODRIGUES, T. et al. GLP-1 improves adipose tissue glyoxalase activity and capillarization improving insulin sensitivity in type 2 diabetes. **Pharmacological Research**, v. 161, p. 105198, 1 nov. 2020.

ROESLER, R. et al. Antioxidant activity of Caryocar brasiliense (pequi) and characterization of components by electrospray ionization mass spectrometry. **Food Chemistry**, v. 110, n. 3, p. 711–717, 1 out. 2008.

ROGACKA, D.; PIWKOWSKA, A. Beneficial effects of metformin on glomerular podocytes in diabetes. **Biochemical Pharmacology**, v. 192, p. 114687, 1 out. 2021.

ROSCOE, R.; RICHETTI, A.; MARANHO, E. Análise de viabilidade técnica de oleaginosas para produção de biodiesel em Mato Grosso do Sul. **Revista de Política Agrícola**, v. 16, n. 1, p. 48-59–59, 2007.

RUEGENBERG, S. et al. Loss of GFAT-1 feedback regulation activates the hexosamine pathway that modulates protein homeostasis. **Nature Communications**, v. 11, n. 1, p. 687, 4 fev. 2020.

RUFINO, M. DO S. M. et al. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. **Food Chemistry**, v. 121, n. 4, p. 996–1002, 15 ago. 2010.

SAADELDEEN, F. S. A. et al. Natural products: Regulating glucose metabolism and improving insulin resistance. **Food Science and Human Wellness**, v. 9, n. 3, p. 214–228, 1 set. 2020.

SANJINEZ-ARGANDOÑA, E. J.; CHUBA, C. A. M. Biometrical, physical and chemical characterization of bocaiuva (Acrocomia aculeata (jacq.) Lodd. ex Mart) Palm fruits. **Revista Brasileira de Fruticultura**, v. 33, n. 3, p. 1023–1028, set. 2011.

SANTOS-BUELGA, C. et al. Chapter Four - Plant phenolics as functional food ingredients. In: FERREIRA, I. C. F. R.; BARROS, L. (Eds.). . **Advances in Food and Nutrition Research**. Functional Food Ingredients from Plants. [s.l.] Academic Press, 2019. v. 90p. 183–257.

SARAIVA, M. E. et al. Plant species as a therapeutic resource in areas of the savanna in the state of Pernambuco, Northeast Brazil. **Journal of Ethnopharmacology**, v. 171, p. 141–153, 2 ago. 2015.

SAYED, A. M. et al. Flavonoids-mediated SIRT1 signaling activation in hepatic disorders. **Life Sciences**, v. 259, p. 118173, 15 out. 2020.

SCHARTNER, E. et al. High glucose concentration suppresses a SIRT2 regulated pathway that enhances neurite outgrowth in cultured adult sensory neurons. **Experimental Neurology**, v. 309, p. 134–147, 1 nov. 2018.

SERAGLIO, S. K. T. et al. Effect of in vitro gastrointestinal digestion on the bioaccessibility of phenolic compounds, minerals, and antioxidant capacity of Mimosa scabrella Bentham honeydew honeys. **Food Research International**, v. 99, p. 670–678, 1 set. 2017.

SERPELONI, J. M. et al. Antimutagenicity and induction of antioxidant defense by flavonoid rich extract of Myrcia bella Cambess. in normal and tumor gastric cells. **Journal of Ethnopharmacology**, v. 176, p. 345–355, 24 dez. 2015.

SHABALALA, S. et al. Polyphenols, autophagy and doxorubicin-induced cardiotoxicity. **Life Sciences**, v. 180, p. 160–170, 1 jul. 2017.

SIES, H. Chapter 13 - Oxidative Stress: Eustress and Distress in Redox Homeostasis. In: FINK, G. (Ed.). . **Stress: Physiology, Biochemistry, and Pathology**. [s.l.] Academic Press, 2019. p. 153–163.

SIGMA-ALDRICH; MERCK. **Sigma-Aldrich|Merck**. Disponível em: <https://www.sigmaaldrich.com/brazil.html>. Acesso em: 3 maio. 2021.

SILVA, C. P. DA et al. Identification and action of phenolic compounds of Jatobá-do-cerrado (Hymenaea stignocarpa Mart.) on α-amylase and α-glucosidase activities and flour effect on

glycemic response and nutritional quality of breads. **Food Research International**, v. 116, p. 1076–1083, 1 fev. 2019.

SILVA, P. **Caracterização Química e avaliação do Potencialantidiabético e Citotóxico de Oleo Extraído de Acrocomia aculeata (macaúba)**. [s.l.] Master's thesis. Universidade Federal da Grande Dourados, Dourados, 2012.

SINGH, V. P. et al. Advanced Glycation End Products and Diabetic Complications. **The Korean Journal of Physiology & Pharmacology : Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology**, v. 18, n. 1, p. 1–14, fev. 2014.

SIQUEIRA, E. M. DE A. et al. Consumption of baru seeds [Dipteryx alata Vog.], a Brazilian savanna nut, prevents iron-induced oxidative stress in rats. **Food Research International**, v. 45, n. 1, p. 427–433, 1 jan. 2012.

SOFOWORA, A.; OGUNBODEDE, E.; ONAYADE, A. The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. **African Journal of Traditional, Complementary, and Alternative Medicines**, v. 10, n. 5, p. 210–229, 12 ago. 2013.

SOUZA, G. K. et al. Synthesis of ethyl esters from crude macauba oil (Acrocomia aculeata) for biodiesel production. **Fuel**, v. 165, p. 360–366, 1 fev. 2016.

SREĆKOVIĆ, N. et al. Lythrum salicaria L. (Lythraceae) as a promising source of phenolic compounds in the modulation of oxidative stress: Comparison between aerial parts and root extracts. **Industrial Crops and Products**, v. 155, p. 112781, 1 nov. 2020.

SUBRAMANIAM, M. D. et al. Oxidative stress and mitochondrial transfer: A new dimension towards ocular diseases. **Genes & Diseases**, 5 dez. 2020.

SULAIMAN, C. T.; BALACHANDRAN, I. Total Phenolics and Total Flavonoids in Selected Indian Medicinal Plants. **Indian Journal of Pharmaceutical Sciences**, v. 74, n. 3, p. 258–260, 2012.

TAFOLLA-ARELLANO, J. C. et al. Composición, fisiología y biosíntesis de la cutícula en plantas. **Revista fitotecnia mexicana**, v. 36, n. 1, p. 3–12, mar. 2013.

TAN, B. L.; NORHAIZAN, M. E. Effect of High-Fat Diets on Oxidative Stress, Cellular Inflammatory Response and Cognitive Function. **Nutrients**, v. 11, n. 11, p. 2579, 25 out. 2019.

TAYLOR, O. M.; LAM, C. The Effect of Dipeptidyl Peptidase-4 Inhibitors on Macrovascular and Microvascular Complications of Diabetes Mellitus: A Systematic Review. **Current Therapeutic Research**, v. 93, p. 100596, 1 jan. 2020.

TAYMAZ-NIKEREL, H. et al. Doxorubicin induces an extensive transcriptional and metabolic rewiring in yeast cells. **Scientific Reports**, v. 8, n. 1, p. 13672, 12 set. 2018.

TEIXEIRA, D. A. et al. A rapid enzyme-catalyzed pretreatment of the acidic oil of macauba (Acrocomia aculeata) for chemoenzymatic biodiesel production. **Process Biochemistry**, v. 53, p. 188–193, 1 fev. 2017.

TEIXEIRA, G. P.; FARIA, R. X. Influence of purinergic signaling on glucose transporters: A possible mechanism against insulin resistance? **European Journal of Pharmacology**, v. 892, p. 173743, 5 fev. 2021.

TENG, Z.; SHEN, Y. [Research progress of genetic engineering on medicinal plants]. **Zhongguo Zhong Yao Za Zhi = Zhongguo Zhongyao Zazhi = China Journal of Chinese Materia Medica**, v. 40, n. 4, p. 594–601, fev. 2015.

THORN, C. F. et al. Doxorubicin pathways: pharmacodynamics and adverse effects. **Pharmacogenetics and Genomics**, v. 21, n. 7, p. 440–446, jul. 2011.

TOMIOTTO-PELLISSIER, F. et al. Caryocar coriaceum extracts exert leishmanicidal effect acting in promastigote forms by apoptosis-like mechanism and intracellular amastigotes by Nrf2/HO-1/ferritin dependent response and iron depletion: Leishmanicidal effect of Caryocar coriaceum leaf exracts. **Biomedicine & Pharmacotherapy**, v. 98, p. 662–672, 1 fev. 2018.

TORGOVNICK, A.; SCHUMACHER, B. DNA repair mechanisms in cancer development and therapy. **Frontiers in Genetics**, v. 6, p. 157, 23 abr. 2015.

TOYOKUNI, S. et al. Serum 4-hydroxy-2-nonenal-modified albumin is elevated in patients with type 2 diabetes mellitus. **Antioxidants & Redox Signaling**, v. 2, n. 4, p. 681–685, 2000.

TRINDADE DE PAULA, M. et al. High-Fat Diet Induces Oxidative Stress and MPK2 and HSP83 Gene Expression in Drosophila melanogaster. **Oxidative Medicine and Cellular Longevity**, v. 2016, p. e4018157, 7 ago. 2016.

TRUJILLO, J. M.; NUFFER, W.; ELLIS, S. L. GLP-1 receptor agonists: a review of head-to-head clinical studies. **Therapeutic Advances in Endocrinology and Metabolism**, v. 6, n. 1, p. 19–28, fev. 2015.

TUNGMUNNITHUM, D. et al. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. **Medicines**, v. 5, n. 3, 25 ago. 2018.

TURK, Z.; MESIĆ, R.; BENKO, B. Comparison of advanced glycation endproducts on haemoglobin (Hb-AGE) and haemoglobin A1c for the assessment of diabetic control. **Clinica Chimica Acta**, v. 277, n. 2, p. 159–170, 1 out. 1998.

TURRENS, J. F. Mitochondrial formation of reactive oxygen species. **The Journal of Physiology**, v. 552, n. Pt 2, p. 335–344, 15 out. 2003.

VALKO, M. et al. Free radicals, metals and antioxidants in oxidative stress-induced cancer. **Chemico-Biological Interactions**, v. 160, n. 1, p. 1–40, 10 mar. 2006.

VASCONCELOS, S. M. L. et al. Espécies reativas de oxigênio e de nitrogênio, antioxidantes e marcadores de dano oxidativo em sangue humano: principais métodos analíticos para sua determinação. **Química Nova**, v. 30, n. 5, p. 1323–1338, out. 2007.

VATNER, S. F. et al. Healthful aging mediated by inhibition of oxidative stress. **Ageing Research Reviews**, v. 64, p. 101194, 1 dez. 2020.

VIIGIMAA, M. et al. Macrovascular Complications of Type 2 Diabetes Mellitus. **Current Vascular Pharmacology**, v. 18, n. 2, p. 110–116, 2020.

VILLA, S. R. et al. Loss of Free Fatty Acid Receptor 2 leads to impaired islet mass and beta cell survival. **Scientific Reports**, v. 6, n. 1, p. 28159, 21 jun. 2016.

WALLACE, K. B.; SARDÃO, V. A.; OLIVEIRA, P. J. Mitochondrial Determinants of Doxorubicin-Induced Cardiomyopathy. **Circulation Research**, v. 126, n. 7, p. 926–941, 27 mar. 2020.

WANG, D. et al. Green tea polyphenols mitigate the plant lectins-induced liver inflammation and immunological reaction in C57BL/6 mice via NLRP3 and Nrf2 signaling pathways. **Food and Chemical Toxicology**, v. 144, p. 111576, 1 out. 2020.

WAYPA, G. B.; SCHUMACKER, P. T. Oxygen sensing in hypoxic pulmonary vasoconstriction: using new tools to answer an age-old question. **Experimental Physiology**, v. 93, n. 1, p. 133–138, 2008.

WHO (ED.). **WHO monographs on selected medicinal plants**. Geneva: World Health Organization, 1999.

WHO. **WHO Monographs on Selected Medicinal Plants, Vol. 2**. Geneva: [s.n.].

WHO. **WHO Monographs On Selected Medicinal Plants - Volume 3**. Disponível em: <https://www.medbox.org/document/who-monographs-on-selected-medicinal-plants-volume-3#GO>. Acesso em: 2 maio. 2021.

WHO. **WHO Monographs On Selected Medicinal Plants - Volume 4**. Disponível em: <https://www.medbox.org/document/who-monographs-on-selected-medicinal-plants-volume-4#GO>. Acesso em: 2 maio. 2021.

WHO. (World Health Organization - 2011 The World Traditional Medicines Situation, in Traditional medicines: Global Situation, Issues and Challenges. Geneva 3:1–14. 2011.

WHO. **WHO reveals leading causes of death and disability worldwide: 2000-2019**. Disponível em: <https://www.who.int/news/item/09-12-2020-who-reveals-leading-causes-of-death-and-disability-worldwide-2000-2019>. Acesso em: 29 abr. 2021.

WHO. **Diabetes**. Disponível em: <https://www.who.int/news-room/fact-sheets/detail/diabetes>. Acesso em: 29 abr. 2021a.

WHO. **Cancer**. Disponível em: <https://www.who.int/news-room/fact-sheets/detail/cancer>. Acesso em: 29 abr. 2021b.

WHO. **Cardiovascular diseases**. Disponível em: <https://www.who.int/westernpacific/health-topics/cardiovascular-diseases>. Acesso em: 11 out. 2021c.

XIU-QIN, L. et al. **Analysis of synthetic antioxidants and preservatives in edible vegetable oil by HPLC/TOF-MS, Food Chem**, 2009.

YAGISHITA, Y. et al. Nrf2 Improves Leptin and Insulin Resistance Provoked by Hypothalamic Oxidative Stress. **Cell Reports**, v. 18, n. 8, p. 2030–2044, fev. 2017.

YAMAMOTO, Y.; GAYNOR, R. B. Role of the NF-kappaB pathway in the pathogenesis of human disease states. **Current Molecular Medicine**, v. 1, n. 3, p. 287–296, jul. 2001.

YAN, J. et al. Characteristics and mechanism of simultaneous nitrate and phenol removal by a newly isolated Cupriavidus oxalaticus T2. **International Biodeterioration & Biodegradation**, v. 161, p. 105234, 1 jul. 2021.

YAN, L. Redox imbalance stress in diabetes mellitus: Role of the polyol pathway. **Animal Models and Experimental Medicine**, v. 1, n. 1, p. 7–13, 19 abr. 2018.

ZHANG, C. et al. MafA Is a Key Regulator of Glucose-Stimulated Insulin Secretion. **Molecular and Cellular Biology**, v. 25, n. 12, p. 4969–4976, jun. 2005.

ZHANG, H.; WANG, L.; CHU, Y. Reactive oxygen species: The signal regulator of B cell. **Free Radical Biology and Medicine**, Oxidative Stress in the Perinatal Period. v. 142, p. 16–22, 1 out. 2019.

ZHANG, Y. et al. ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages. **Cell Research**, v. 23, n. 7, p. 898–914, jul. 2013.

ZHAO, L.; ZHANG, B. Doxorubicin induces cardiotoxicity through upregulation of death receptors mediated apoptosis in cardiomyocytes. **Scientific Reports**, v. 7, n. 1, p. 44735, 16 mar. 2017.

ZHAO, Z. et al. Age-Related Retinopathy in NRF2-Deficient Mice. **PLOS ONE**, v. 6, n. 4, p. e19456, 29 abr. 2011.

ZHOU, F. et al. Protective effect of 23-hydroxybetulinic acid on doxorubicin-induced cardiotoxicity: a correlation with the inhibition of carbonyl reductase-mediated metabolism. **British Journal of Pharmacology**, v. 172, n. 23, p. 5690–5703, dez. 2015.

5 APPENDIX

Paper I

Hypoglycaemic and Antioxidant Properties of *Acrocomia aculeata* (Jacq.) Lodd Ex Mart. Extract Are Associated with Better Vascular Function of Type 2 Diabetic Rats

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Article

Hypoglycaemic and Antioxidant Properties of *Acrocomia aculeata* (Jacq.) Lodd Ex Mart. Extract Are Associated with Better Vascular Function of Type 2 Diabetic Rats

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Abstract: Oxidative stress is involved in the metabolic dysregulation of type 2 diabetes (DM2). *Acrocomia aculeata* (Aa) fruit pulp has been described for the treatment of several diseases, and recently we have proved that its leaves have phenolic compounds with a marked antioxidant effect. We aimed to assess whether they can improve metabolic, redox and vascular functions in DM2. Control Wistar (W-Ctrl) and non-obese type 2 diabetic Goto–Kakizaki (GK-Ctrl) rats were treated for 30 days with 200 mg.kg^{-1} aqueous extract of Aa (EA-Aa) (Wistar, W-EA-Aa/GK, GK-EA-Aa). EA-Aa was able to reduce fasting glycaemia and triglycerides of GK-EA-Aa by improving proteins related to glucose and lipid metabolism, such as GLUT-4, PPARγ, AMPK, and IR, when compared to GK-Ctrl. It also improved viability of 3T3-L1 pre-adipocytes exposed by H_2O_2. EA-Aa also increased the levels of catalase in the aorta and kidney, reduced oxidative stress and increased relaxation of the aorta in GK-treated rats in relation to GK-Ctrl, in addition to the protective effect against oxidative stress in HMVec-D cells. We proved the direct antioxidant potential of the chemical compounds of EA-Aa, the increase in antioxidant defences in a tissue-specific manner and hypoglycaemic properties, improving vascular function in type 2 diabetes. EA-Aa and its constituents may have a therapeutic potential for the treatment of DM2 complications.

Keywords: diabetes; *macaúba*; *bocaiúva*; vascular function; polyphenols

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1. Introduction

Diabetes mellitus (DM) is a chronic disease characterized by hyperglycaemia, resulting from a deficiency in insulin production, desensitization of its action, or both [1]. According to the International Diabetes Federation, diabetes is one of the diseases with the highest incidence in the 21st century, having increased three-fold in the last two decades, and being estimated to affect 463 million individuals in 2019 [2]. As a consequence of hyperglycaemia, both DM1 and DM2 commonly have associated complications, which have significant morbidity and mortality and a considerable economic impact. These complications can be either micro (neuro, nephro, cardio and retinopathy) or macro vascular (stroke and cardiovascular diseases) [3,4]. One of the main factors for its cause and progression is oxidative stress, which is involved in the pathogenesis of complications through the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), further impairing the redox balance [5]. The adoption of a better lifestyle (balanced diet and exercise) may delay the development of diabetes and its complications. On the other hand, several therapeutic options are available for DM2, most of them targeted for the reduction of the glycaemia [meglitinides, biguanides, sulfonylureas (SUs), thiazolidinedione (TZD), dipeptidyl peptidase 4 (DPP-4) inhibitors, GLP-1 receptor agonists, sodium glucose cotransporters inhibitors (SGLT2) and insulin] [6]. However, a considerable number of patients do not adhere to the treatment with allopathic medicine due to side effects [4], which affect mainly liver and kidney [7]. In this perspective, a considerable amount of research is focused on developing therapeutic alternatives, which could be both inexpensive and effective with fewer side effects [4].

Brazil has the greatest biodiversity in the world, with about 20% of the species distributed in its biomes. In particular, the Brazilian Cerrado, which occupies 22% of the national territory [8], has one of the world's richest flora, where 35% of the species are endemic [9]. Among these species, many of them are considered medicinal due to their chemical composition [10]. *Acrocomia aculeata* (Jacq.) Lodd. ex Mart., commonly known as *bocaiúva* or *macaúba*, is a palm native from Cerrado with therapeutic (production of remedies based on ethnopharmacological knowledge—as antidiabetic, antioxidant, analgesic etc.) and economic importance (for cooking, biodiesel, and the cosmetic industry) [11]. Its carotenoid-rich fruit pulp was suggested to have beneficial effects on the treatment of respiratory diseases, as analgesic and laxative [12] and also in decreasing serum cholesterol and glucose levels [13]. Recently, our group proved the antioxidant potential of its leaves and the relevant chemical composition, mostly of vanillic, caffeic, ferulic and gallic acid, rutin and quercetin [11]. In addition to the new findings described by us about the potential of its leaves, it is only known that they are used for bovine nutritional supplementation and in the preparation of teas for human consumption.

Therefore, our goal in this study was to assess whether the antioxidant potential of *A. aculeata* leaves can restore redox balance and improve metabolic and vascular function of type 2 diabetic rats, as well as to disclose the underlying mechanisms in tissues involved in glucose metabolism and its vascular complications. Our results show the antioxidant and hypoglycaemic potential of EA-Aa, observed through the tissue-dependent upregulation of pathways involved in antioxidant defences and glucose and lipid metabolism. Such effects were associated with the improvement of aortic relaxation and redox state.

2. Materials and Methods

2.1. Chemicals and Antibodies

Salts and organic solvents used in this study were all purchased from Lonza, Sigma-Aldrich/Merck, Alfa-Aesar, Fischer Scientifics and Panreac. Antibodies used were targeted to Catalase, Glo-1, GLUT2 (ab76110, ab96032, ab54460 Abcam, Cambridge, UK), GLUT4, PPARgamma, Insulin Receptor, AMPK, phospho-AMPK-Thr-172, Sirt1, phospho-Sirt1-Ser47 (#2213S, #2443S, #3025S, #2532S, #2535S, #9475S, #2314S, Cell Signaling Technology, Danvers, MA, USA) NRF2 (sc-518036, Santa Cruz Biotechnology, Dallas, TX, USA) phospho-

NRF2 (Ser40) (PA5-67520, Invitrogen, Waltham, MA, USA). Calnexin and GAPDH (AB0037, AB0049-20, Sicgen, Carcavelos, Portugal) were used as loading control.

2.2. Botanical Material and Isolation of Extract

Fresh *A. aculeata* leaves were collected as before [11] in the region of Grande Dourados, Macaúba district, state of Mato Grosso do Sul (MS) (22°0702.4 S 54°2836.3 W), with the permission of the Brazilian Biodiversity Authorization and Information System (*Sistema de Autorização e Informação sobre Biodiversidade*, SISBIO; no. 50589). A plant taxonomist identified the species, and a specimen was deposited in the herbarium (DDMS-UFGD) of the Federal University of Grande Dourados, Dourados (MS), Brazil, registration number—5103. The aqueous extract was prepared as previously described [11].

2.3. Cell Culture and Viability Assays

Mouse (*Mus musculus*) preadipocyte—3T3-L1 cells (cultured with Dulbecco's Modified Eagle's Medium—DMEM supplemented with 10% FBS and 1% penicillin/streptomycin) [14]; and human dermal microvascular endothelial Cells (HMVec-D, cultured with EGMTM-2, Endothelial Cell Growth Medium-2, BulletKitTM) [15,16], maintained at 37 °C and 5% CO_2 were used in the assays.

To evaluate cell viability, 1×10^5 HMVec-D cells and 3×10^4 3T3-L1 cells were seeded in 96-well microplates. After 24 h, cells were incubated with different concentrations (31,25–500 $\mu g.mL^{-1}$) of EA-Aa for 24 h. After this period, cell viability was determined through the Alamar Blue assay. Absorbance was measured at 570 nm and 600 nm in a BioTek microplate reader (BioTek, Instruments, Inc., Winooski, VT, USA) and used to calculate cell viability, according to Equation (1) [11].

$$\text{Cell viability} = \left(\frac{(\text{Abs}_{570} - \text{Abs}_{600})\text{of treated cells}}{(\text{Abs}_{570} - \text{Abs}_{600})\text{of control cells}} \right) \times 100 \quad (1)$$

To evaluate the antioxidant potential of EA-Aa, both cell lines 3T3-L1 and HMVec-D cells were treated with H_2O_2, the oxidative stress inductor. After 80% of confluence, cells were firstly incubated with the extract for 30 min followed by H_2O_2 (IC_{50} 0.125 mM in 3T3-L1 cells and 0.25 mM in HMVec-D cells) for 2 h. Equation (1) was used to calculate the protective effect of EA-Aa in cell viability. Dependence of EA-Aa effects on NRF2 pathway was evaluated through the incubation of HMVec-D cells with the NRF2 inhibitor ML385 (20 μM).

2.4. Animal Maintenance and Treatment

The study was performed according to good practices of animal handling, with the approval of the Institutional Animal Care and Use Committee (ORBEA 13/2018) and the procedures performed by licensed users by the Federation of Laboratory Animal Science Associations (FELASA), conformed to the guidelines from Directive 2010/63/EU of the European Parliament for the Protection of Animals Used for Science Purpose. Male 12-week-old Wistar and non-obese type 2 diabetic Goto–Kakizaki (GK) rats from our breeding colonies (Faculty of Medicine, University of Coimbra), were randomly divided in 4 groups (n = 5–7), as presented in Figure 1A, which were: Wistar control (W); Wistar treated with EA-Aa (W-EA-Aa); GK control (GK) and GK treated with EA-Aa (GK-EA-Aa). Animals were kept under standard conditions—2 animals per cage, with temperature at 22–24 °C, and 50–60% humidity, and standard light cycle (12 h light/12 h darkness), with water and food (standard diet A03, SAFE, France) ad libitum [17]. EA-Aa (200 $mg.kg^{-1}$) was added in the daily water of the animals 28 days, which received the treatment during the night and normal water during the day. The weekly average of the rats' weight was used to determine the daily dose of EA-Aa per cage.

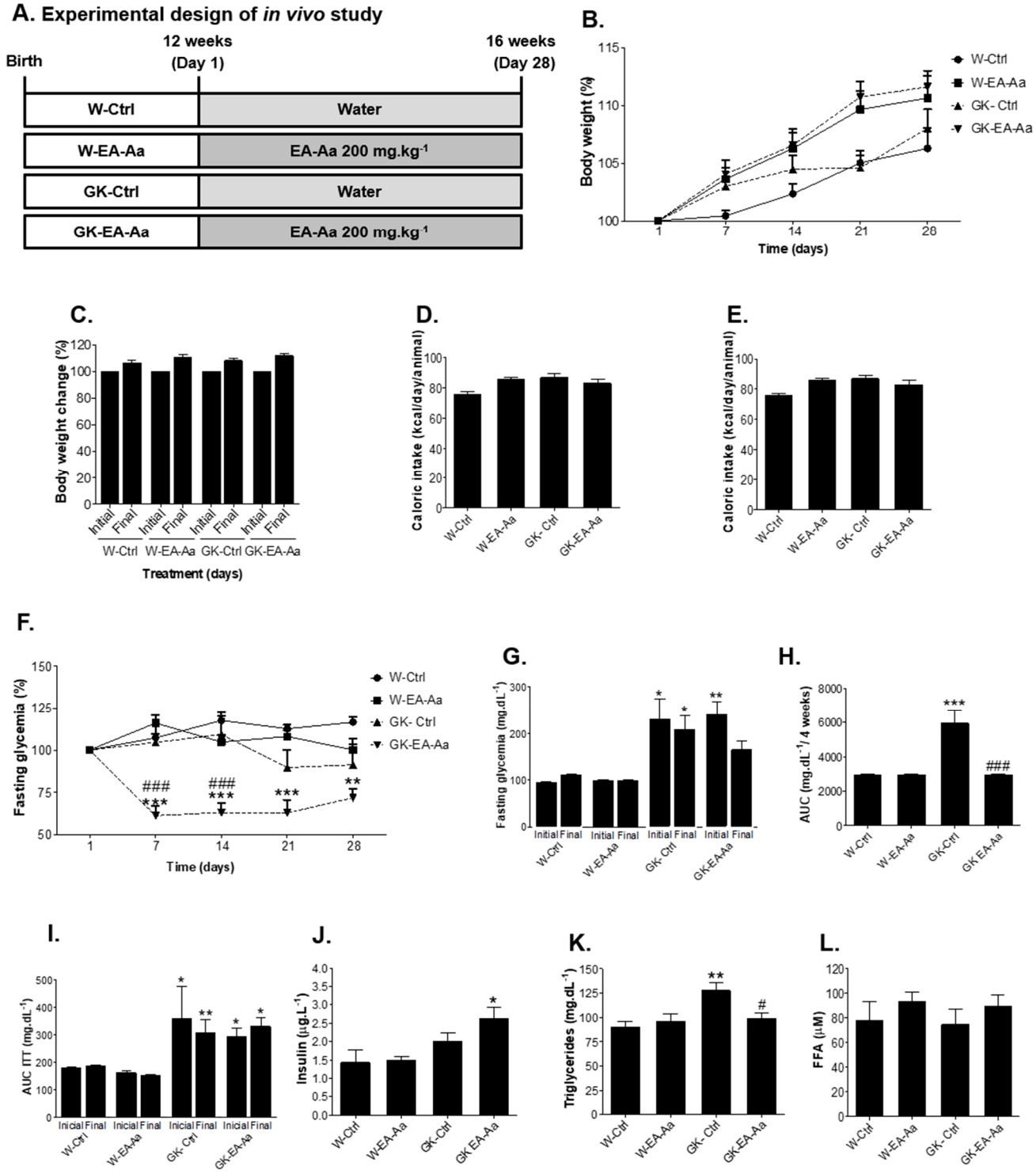

Figure 1. Glycaemic and lipid profile of Wistar and Goto–Kakizaki (GK) rats after 30-day treatment (n = 5–7). (**A**) Experimental design of in vivo study. (**B**) Body mass evolution of treated and non-treated rats represented every 7 days. (**C**) Initial and final body mass of treated animals. (**D**) Caloric intake. (**E**) Fasting blood glucose in percentage of the initial value; EA-Aa promoted a decrease in fasting glycaemia of GK-EA-Aa since the 7th day of treatment in relation to the initial glycaemia. (**F**) Fasting glycaemia; a restoration of values is found in the end of the treatment between GK rats and W-Ctrl group. (**G**) Area under the curve of glycaemia along 4-week treatment period; values are reduced by EA-Aa treatment in GK rats when comparing to the control group. (**H**) Area Under the curve of the glycaemia along all the treatment. (**I**) Area under the curve of ITT (insulin tolerance test). (**J**) Insulin; an increase in GK-EA-Aa is presented in relation to the W-Ctrl group. (**K**) Triglycerides; treatment with EA-Aa decreased plasma triglycerides levels in GK. (**L**) Free fatty acid levels. * vs. W-Ctrl at the same point; # vs. GK-Ctrl at the same point; *,# $p < 0.05$; ** $p < 0.01$; ***,### $p < 0.001$.

2.4.1. In Vivo Procedures and Sample Collection

Body weight, fasting glycaemia, water and food intake were evaluated weekly (calculated as the mean daily consumption per rat), and an insulin tolerance test (ITT) was performed at the beginning and at the end of the treatment. For the IIT, 250 $mU.kg^{-1}$ insulin (Humulin, 1000 $UI.mL^{-1}$ Lilly, Lisboa, Portugal) was injected (i.p.) after 6 h fasting, followed by glycaemia measurement in the tail vein with a glucometer (Precision Xtra Meter, Abbott Diabetes Care, Amadora, Portugal) and test strips (Abbott Diabetes Care, Portugal) at time 0, 15, 30, 60 and 120 min. Response to insulin was expressed by area under the curve (AUC) [17]. Serum triglycerides were measured in the same day before insulin administration. At the end of the treatment, animals were anesthetized (i.p.) with 2:1 (*v*/*v*) 50 $mg.kg^{-1}$ ketamine (100 $mg.mL^{-1}$)/2.5% chlorpromazine (5 $mg.mL^{-1}$) and samples of blood were collected by cardiac puncture followed by cervical dislocation. Epididymal adipose tissue (EAT), liver, kidney, heart and aorta were collected, blood samples were centrifuged (2200× *g*, 4 °C, 15′) and serum and plasma were aliquoted and stored at −80 °C for further analysis.

2.4.2. Studies of Isometric Tension of Aorta

Aorta rings were mounted on stainless steel hooks under 19.6 mN basal tension in organ baths filled with aerated (95% O_2, 5% CO_2) Krebs–Henseleit solution (37 °C, pH 7.4) (NaCl 118.67 mmol/L; KCl 5.36 mmol/L; $CaCl_2$ 1.90 mmol/L; $MgSO_4$ 0.57 mmol/L; $NaHCO_3$ 25.00 mmol/L; $KH_2PO_4.H_2O$ 0.90 mmol/L; glucose 11.1 mmol/L). After an equilibration period of 60 min, aortic rings were precontracted with 10 µM of noradrenaline and cumulative isometric concentration-response curves were performed in response to acetylcholine (ACh) (0.01 to 90 µM) in the presence and absence of 100 µM ascorbic acid. Cumulative curves were recorded with Letica Scientific Instruments isometric transducers connected to a four-channel polygraph (Polygraph 4006, Letica Scientific Instruments, Barcelona, Spain).

2.5. Biochemical Analyses

Plasma insulin and free fatty acids (FFA) tests were determined through the Rat Insulin ELISA Kit (Mercodia, Uppsala, Sweden) and FFA Assay Kit (ZenBio, Research Triangle Park, NC, USA), according to the manufacturers' instructions. Heart 8-Isoprostane levels were determined using an ELISA Kit according to the manufacturer's instruction (Cayman Chemical, Ann Arbor, MI, USA).

2.6. Fluorescence Immunocytochemistry and Immunohistochemistry

The evaluation of the antioxidant potential of EA-Aa was carried out in HMVec-D cells challenged with H_2O_2 (same as the antioxidant assay described before), and in cryopreserved histological slices (4 µm) of liver, kidney, and aorta of the animal models. Oxidative stress probes, 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA) and dihydroethidium (DHE) were used and DAPI was used to stain the nucleus. In hydrated sections, probes were incubated for 30 min and the slices were mounted with mounting medium (Glycergel, DAKO, Carpinteria, CA, USA). Images were immediately obtained with a fluorescence microscope (Zeiss Axio Observer Z1) with an incorporated camera (Zeiss, Jena, Germany), detected with 504 nm of excitation and 525 nm of emission for DCF, 587 nm of excitation and 610 nm of emission for DHE, and 353 nm of excitation and 465 nm of emission for DAPI. The same settings were kept constant for all analysis and the entire image was used for quantification, which was performed with ImageJ software.

2.7. Western Blot

The Western blot analysis were performed in both cells (3T3-L1 cells treated with EA-Aa 31.25–500 $\mu g.mL^{-1}$ for 24 h) and organs (EAT, heart, kidney, liver and aorta). Cells and organ samples were washed with PBS and disrupted in lysis buffer (0.25 M Tris-HCl, 125 mM NaCl, 1% Triton-X-100, 0.5% SDS, 1 mM EDTA, 1 mM EGTA, 20 mM NaF, 2 mM

Na_3VO_4, 10 mM β-glycerophosphate, 2.5 mM sodium pyrophosphate, 10 mM PMSF, 40 µL of protease inhibitor) using the TissueLyser systems (Quiagen, Germany). The BCA Protein Assay Kit was carried out on the supernatant of the centrifugation of samples (14.000 rpm for 20 min at 4 °C), followed by the addition of Laemmli buffer (62.5 mM Tris-HCl, 10% glycerol, 2% SDS, 5% β-mercaptoethanol, 0.01% bromophenol blue) [18]. Samples (20 µg) were loaded into SDS-PAGE and electroblotted onto PVDF membrane (Advansta, San Jose, CA, USA). Membranes were blocked with TBS-T 0.01% and BSA 5%, then incubated with the primary (overnight, 4 °C) and secondary antibodies (2 h, room temperature), following the dilutions suggested by the manufacturers. Immunoblots were detected with ECL substrate and the Versadoc system (Biorad, Hercules, CA, USA).

2.8. Statistical Analysis

Data were expressed as the mean ± standard error of the mean (SEM) and compared by analysis of variance using the Kruskal–Wallis test or ANOVA followed by the Tukey post hoc test, according to normality evaluation. Student's *t*-test was used to determine the differences between two groups. Values of $p < 0.05$ were considered significant. Statistical tests were performed with GraphPad Prism 5.0 and IBM SPSS Statistics Software.

3. Results

3.1. EA-Aa Improves the Metabolic Profile of Diabetic Rats

After the 30-day treatment (presented in the experimental design, Figure 1A), animals did not show any alterations in body weight and food/caloric intake among groups (Figure 1B–D). However, the treatment with EA-Aa decreased the fasting hyperglycaemia of diabetic rats (GK-EA-Aa) by 30–40% along the treatment in comparison to both controls at all timepoints (Figure 1E). Similar data were observed for fasting glycaemia, where the initial difference between GK-Ctrl and W-Ctrl groups becomes non-significant after the treatment in GK-EA-Aa (Figure 1F), and for the AUC of glycaemia along the 4-week treatment period, which was also significantly reduced in GK-EA-Aa when compared to GK-Ctrl (Figure 1G). No significant effects of EA-Aa were observed in the AUC of the insulin tolerance test (Figure 1H). On the other hand, a significant increase of plasma insulin levels was observed in GK-EA-Aa in relation to W-Ctrl rats (Figure 1I). Regarding lipid metabolism, GK-Ctrl presented higher levels of triglycerides, which were reduced in the GK-EA-Aa group (Figure 1J), whereas the values of FFA showed no significant difference (Figure 1K).

The epididymal adipose tissue and the liver were analysed to understand the mechanisms of EA-Aa-induced metabolic improvement. The haematoxylin-eosin staining presented no morphological alterations and no significant weight changes were observed after EA-Aa treatment in the EAT and liver of Wistar and diabetic rats, as shown in Figure 2A,B,G–H. In addition, GLUT4 and PPARγ levels in EAT were increased in the diabetic rats treated with EA-Aa (Figure 2C,F). No changes were observed for the insulin receptor levels nor AMPK (Figure 2D,E), although a trend to higher AMPK levels was observed in diabetic rats after EA-Aa treatment. In the liver, EA-Aa partially restored the levels of the insulin receptor in GK rats (64% *vs* 38% of %W-Ctrl/Calnexin). No significant differences were observed in hepatic GLUT2 and AMPK levels (Figure 2I,K).

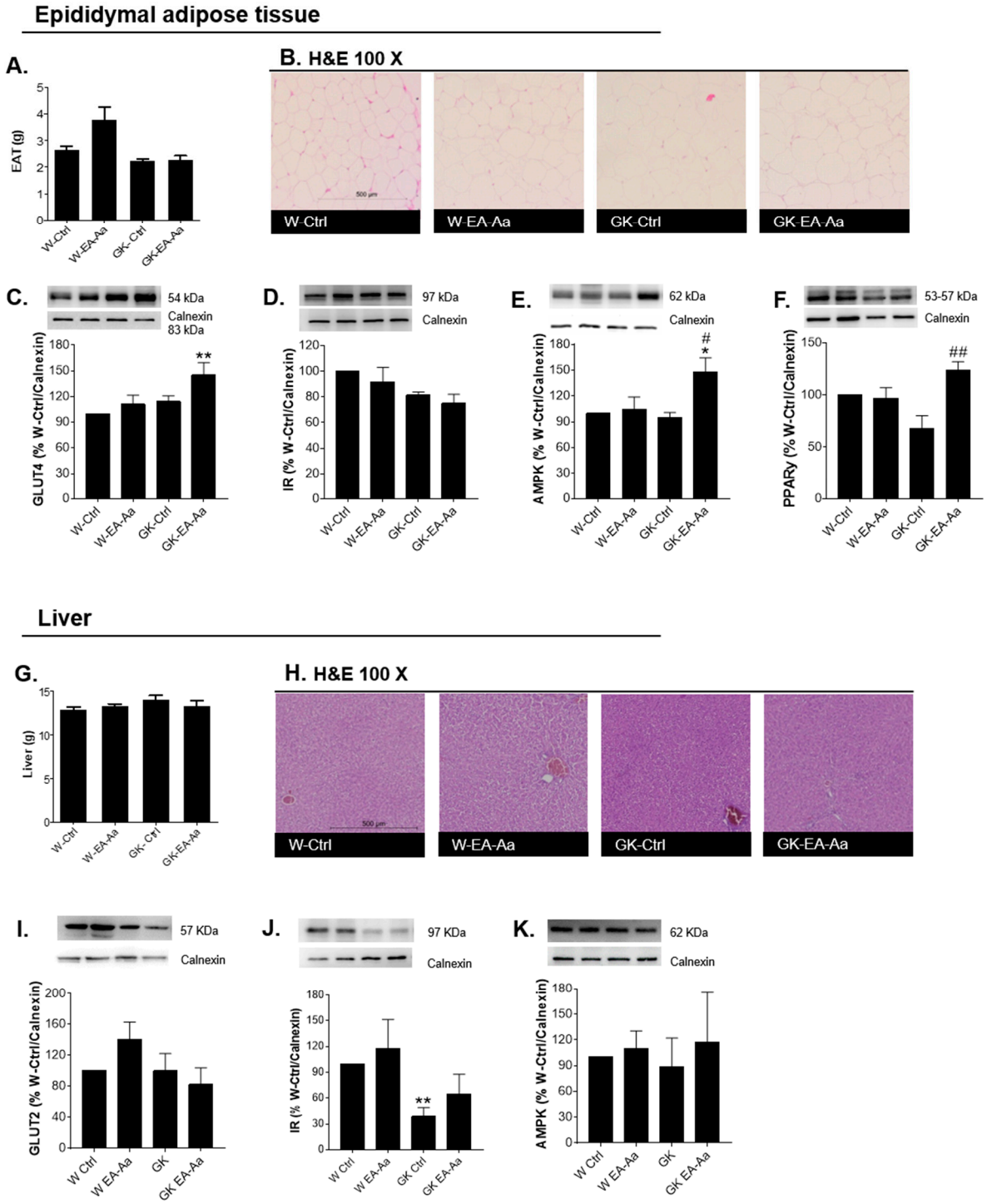

Figure 2. Regulation of metabolic pathways in epididymal adipose tissue (EAT) and liver. (**A**) Epididymal adipose tissue weight (n = 5–7). (**B**) Haematoxylin-eosin staining in EAT (n = 4). (**C**) GLUT4 levels in EAT; treatment with EA-Aa increased the levels of the protein in the treated diabetic group. (**D**) Insulin receptor levels in EAT. (**E**) AMPK levels in EAT; an increase in AMPK levels is presented in EA-Aa GK-treated group in relation to both controls. (**F**) PPARγ levels in EAT; GK-EA-Aa shows an increase in relation to W-Ctrl group (n = 5 for Western blots). (**G**) Liver weight (n = 5–7). (**H**) Haematoxylin-eosin staining in liver (n = 4). (**I**) GLUT2 levels in liver. (**J**) Insulin receptor levels in liver; treatment with EA-Aa promoted a restoration in IR levels in relation to the normal control group. (**K**) AMPK levels in liver (n = 5 for Western blots). * vs. W-Ctrl; # vs. GK-Ctrl; *,# $p < 0.05$; **,## $p < 0.01$.

3.2. EA-Aa Has Tissue-Specific Protective Antioxidant Effects

To determine the antioxidant potential of EA-Aa in vivo, Sirt1, NRF2, catalase, SOD-1 and GLO-1 levels were evaluated in EAT, liver, heart and kidney of control and diabetic rats. Markers of oxidative stress were evaluated and, given the metabolic alterations in adipose tissue caused by EA-Aa, the protective antioxidant effects were evaluated in a 3T3-L1 preadipocyte cell line. In the adipose tissue (Figure 3A,C,D) and liver (Figure 3I–L), no significant differences were observed between control and diabetic rats in Sirt1, catalase, GLO-1 and SOD-1 levels, whereas a partially restoration of NRF2 levels was observed in EAT of GK-EA-Aa group (Figure 3B). In the liver, the histological staining of a superoxide anion probe (DHE) did not show differences between the experimental groups as well (Figure 3H). Given that the antioxidant potential of EA-Aa was already confirmed in Cos-7 cells [11], we evaluated such EA-Aa effect in 3T3-L1 cells challenged with an oxidant stimulus. After confirming the absence of EA-Aa-induced toxicity (Figure 3E), we incubated cells with H_2O_2 (IC_{50}–0.125 mM), which confirmed the protective antioxidant effect of the extract (500 $\mu g./mL^{-1}$) against H_2O_2-induced oxidative stress (Figure 3F). No activation of Sirt1 and NRF2 pathways was observed suggesting that the effect is at least partially independent of such pathways (representative Western Blots at Figure 3G).

Heart and kidney were analysed given their high susceptibility to hyperglycaemia-driven complications. In both organs, no significant alterations were observed in Sirt1, NRF2, GLO-1 and SOD-1 levels (Figure 4A–D,I). Nevertheless, EA-Aa treatment resulted in a significant increase of kidney catalase levels of both normal and diabetic rats (Figure 4H). No significant alterations were observed in the heart levels of the lipid peroxidation marker 8-Isoprostane, as well as in kidney histological analysis of morphology (Figure 4E,G) and superoxide anion (DHE, Figure 4F). A trend to reduced DHE staining in the glomerulus was observed in some kidney regions after EA-Aa treatment, although quantification did not show significant differences (data not shown).

3.3. EA-Aa Improves Diabetic Endothelial Dysfunction Reducing Vascular Oxidative Stress

Given that endothelial dysfunction is one of the major complications of hyperglycaemia, aorta relaxation was evaluated after EA-Aa treatment in Wistar and diabetic rats. Vascular relaxation was evaluated in NA-precontracted aorta rings in response to Ach in the presence or absence of ascorbic acid [19]. At 12-week-old, Wistar and GK rats had similar ACh-dependent relaxation (Figure 5A). In the rats treated with EA-Aa, a slight effect on aorta relaxation was observed, namely a 15% increment between W-EA-Aa and W-Ctrl (Figure 5B) and 10% between GK- EA-Aa and GK-Ctrl (Figure 5C), although statistical significance was not reached. Pre-incubation of the rings with L-NAME (NOS inhibitor) practically abolished the relaxation of the aortic rings (data not shown), showing endothelial dependence. When the aorta was incubated with ascorbic acid, the maximum endothelial-dependent relaxation mediated by NA-precontracted rings in response to Ach reached 40% more in W-Ctrl and almost 74% more in W-EA-Aa (Figure 5D–E). Interestingly, no response to ascorbic acid was obtained in GK-Ctrl (Figure 5F), but it was restored after the treatment with the extract, which promoted a 30% increment of relaxation in GK-EA-Aa (Figure 5G). Such results are supported by the histological fluorescence staining of both oxidative stress probes, DHE and DCF, where 34% and 67% respectively lower signal intensity was observed in GK-EA-Aa in relation to the GK-Ctrl group (Figure 6A–D). The treatment also promoted an increase of catalase levels in the aorta of treated groups from both strains, mainly in diabetic rats (Figure 6E).

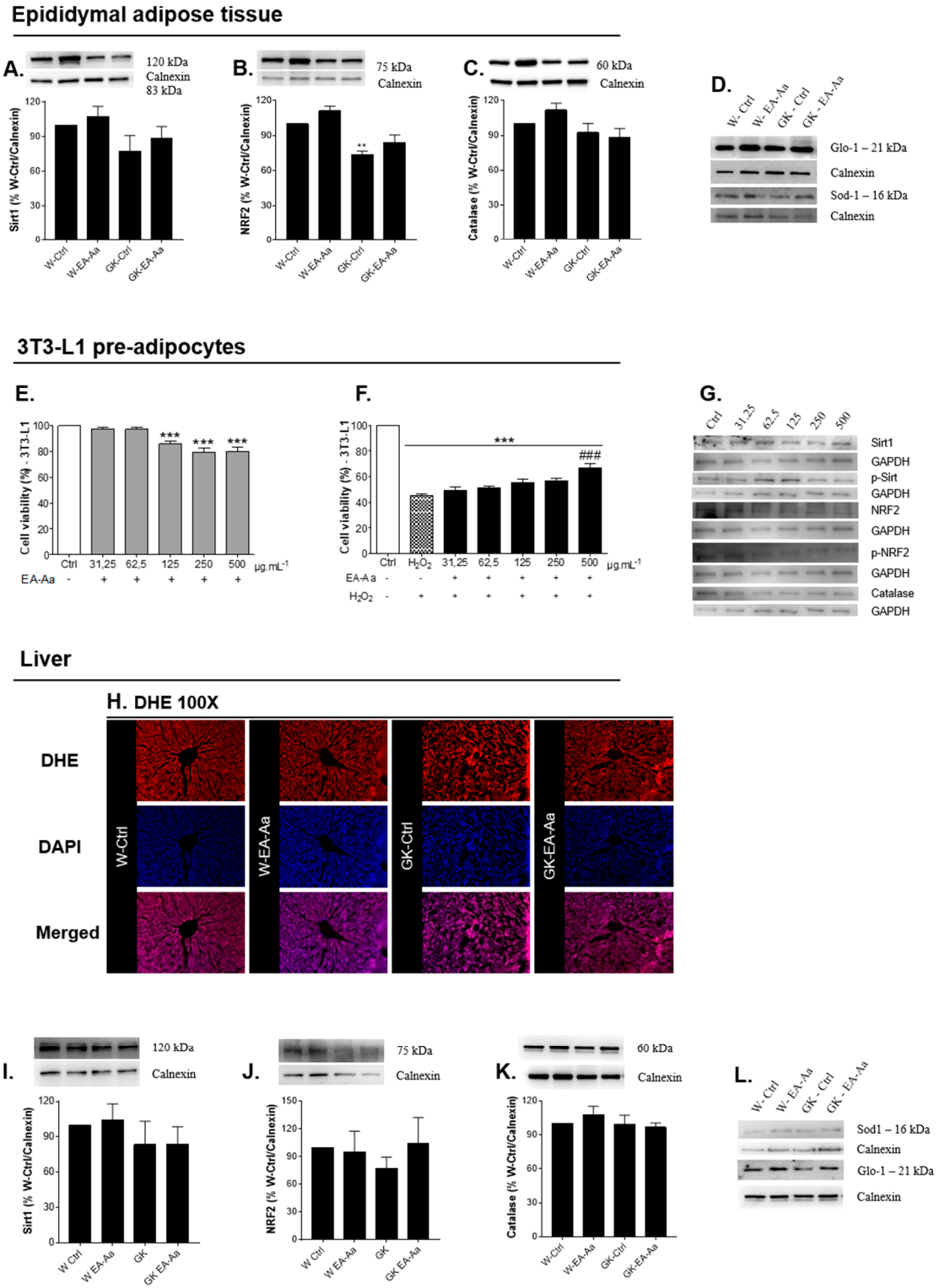

Figure 3. Antioxidant effects of EA-Aa in EAT, liver and in 3T3-L1 pre-adipocytes (n = 3 independent experiments). (**A**) Sirt1 levels in EAT. (**B**) NRF2 levels in EAT, EA-Aa promotes partial restoration of NRF2 levels in the non-obese type 2 diabetic EA-Aa-treated group. (**C**) Catalase levels in EAT. (**D**) Representative images of EAT Western blot. Cell viability of pre-adipocytes 3T3-L1: (**E**) Treatment with EA-Aa for 24 h. (**F**) Treatment with EA-Aa (previously for 30 min) and induction to oxidative stress with H_2O_2 (for 2 h). (**G**) Representative images of 3T3-L1 Western blot. (**H**) Dihydroethidium (DHE) staining in liver (n = 3). (**I**) Sirt1 levels in liver. (**J**) NRF2 levels in liver. (**K**) Catalase levels in liver (n = 5 for Western blots). (**L**) Representative images of liver Western blot. * vs. W-Ctrl/Ctrl; # vs. H_2O_2; ** $p < 0.01$; ***,### $p < 0.001$.

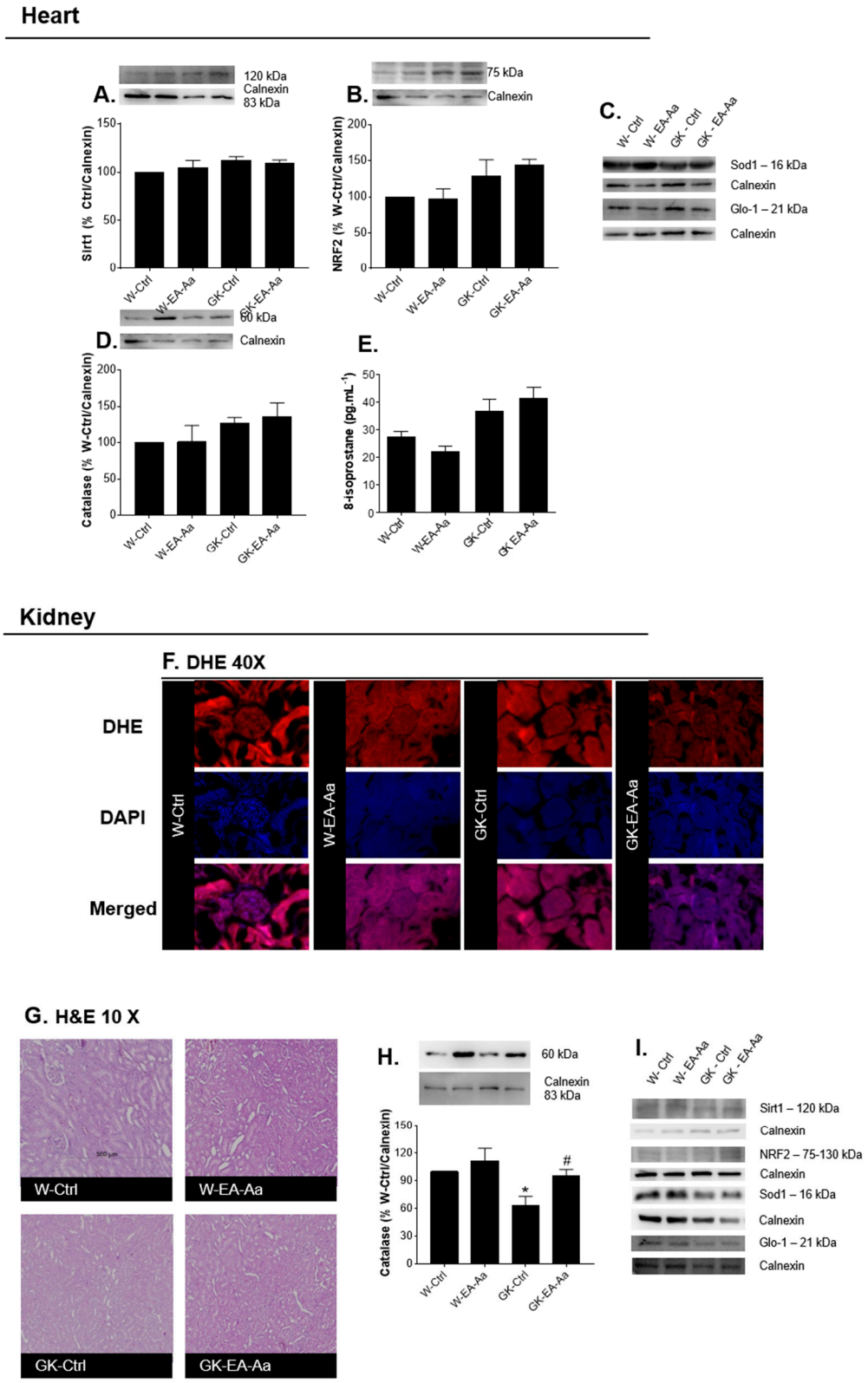

Figure 4. Levels of stress-related proteins in target organs of redox imbalance, heart and kidney. (**A**) Sirt1 levels in heart. (**B**) NRF2 levels in heart. (**C**) Representative images of heart Western blot. (**D**) Catalase levels in heart (n = 5 for Western blots). (**E**) Heart 8-isoprostane levels (n = 5–7). (**F**) DHE staining in kidney (n = 3). (**G**) Haematoxylin-eosin staining in kidney. (**H**) Catalase levels in kidney; a restoration is evident is GK-EA-Aa in relation to GK-Ctrl group. (**I**) Representative images of kidney Western blot. * vs. W-Ctrl/Ctrl; # vs. H_2O_2; *,# $p < 0.05$.

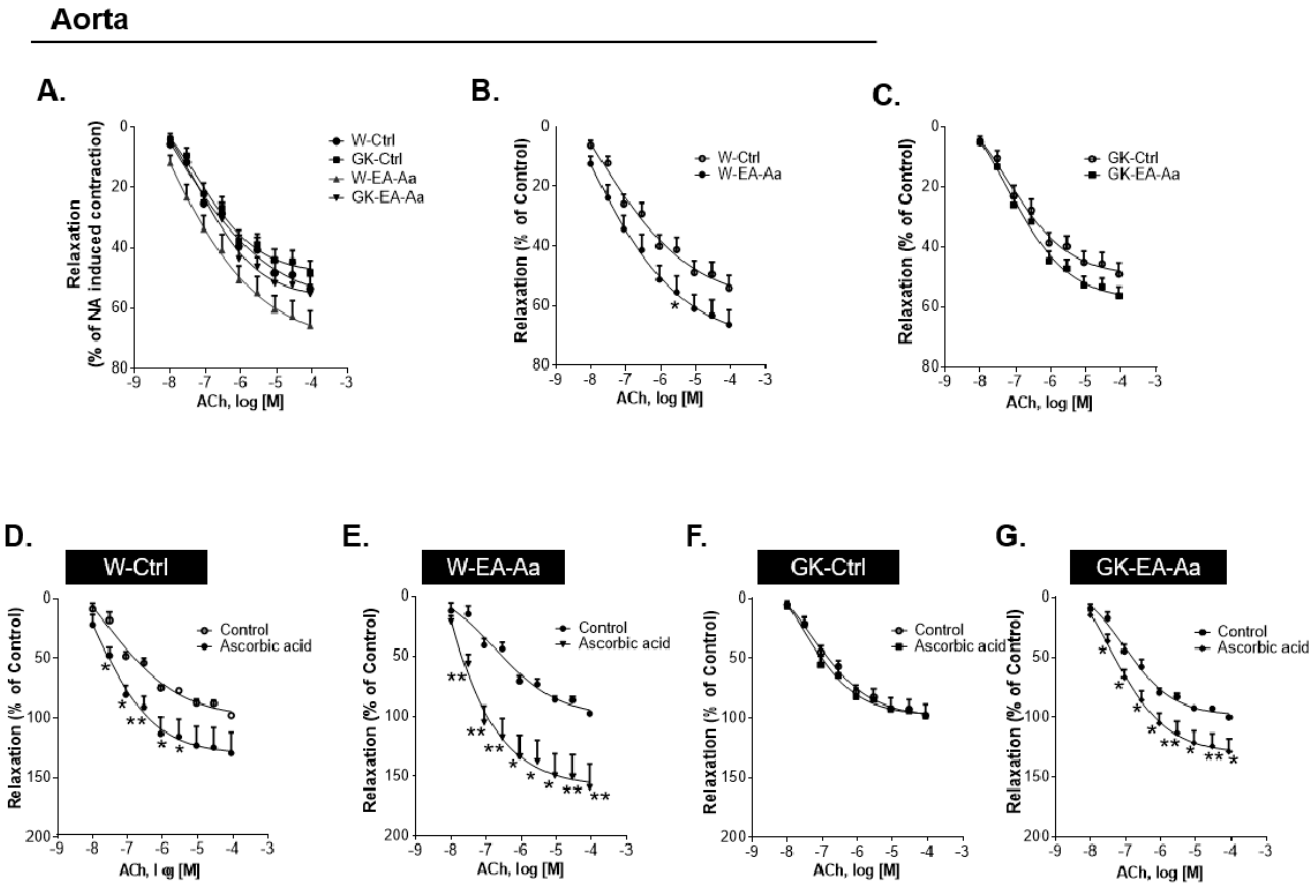

Figure 5. Increased isometric relaxation of aorta promoted by EA-Aa (n = 5-7). Vascular relaxation of NA-precontracted aorta in response to Ach: (**A**) both strains, control and treated rats. (**B**) Wistar group; treatment with EA-Aa shows a light increase in aorta relaxation compared to W-Ctrl. (**C**) GK group. Aorta pre-incubated with ascorbic acid: (**D**) W-Ctrl; an increase in aorta relaxation promoted by ascorbic acid is evident in pre-incubated W-Ctrl. (**E**) W-EA-Aa; treatment with EA-Aa increased the relaxation in normal rats in comparison to the non-pre-incubated group. (**F**) GK-Ctrl. (**G**) GK-EA-Aa; EA-Aa induces an improvement in aorta relaxation in comparison to the non-pre-incubated aorta in diabetic rats. * vs. the same point with or without ascorbic acid pre-incubation; * $p < 0.05$; ** $p < 0.01$.

To further assess the antioxidant effect of EA-Aa in the vessel wall, we used a microvascular endothelial cell line, HMVec-D, in which the absence of toxicity of the EA-Aa can be observed in Figure 6F. In cells with H_2O_2-induced oxidative stress, the EA-Aa had a protective effect in the concentrations of 125, 250 and 500 $\mu g.mL^{-1}$, which is represented by a ~22% improvement of cell viability (Figure 6G). Cells incubated with NRF2 inhibitor, ML385, presented a decrease in cell viability of almost 52% when induced with H_2O_2, which has been improved by EA-Aa treatment in 10% to 20% (according to the increase in concentration). Thus, the protective effects of the EA-Aa were only attenuated in the presence of ML385, suggesting a protection partially mediated by NRF2 pathway, but mostly resulting from the direct antioxidant properties of the extract (Figure 6H).

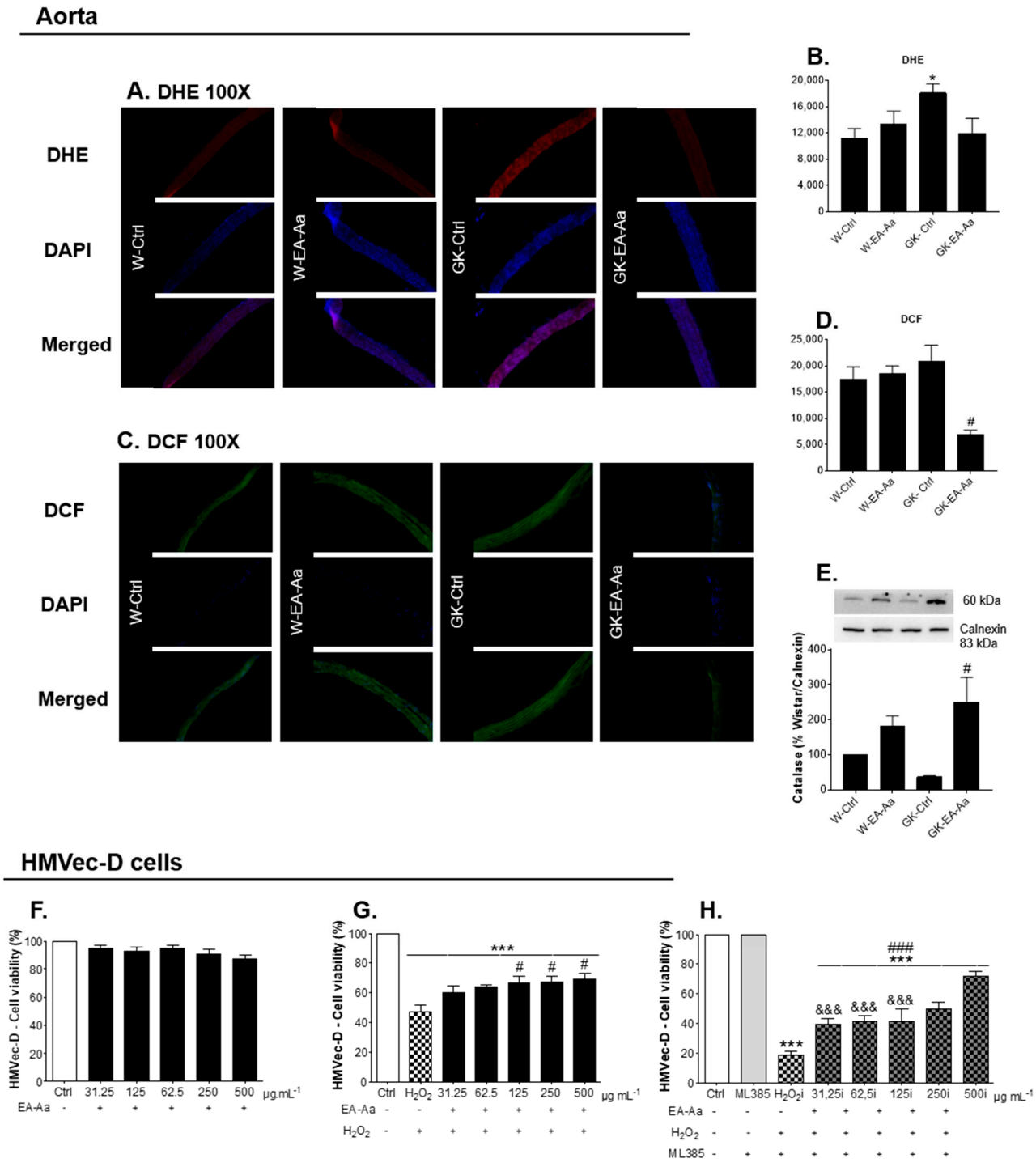

Figure 6. Antioxidant protection of EA-Aa at the vascular level, in aorta and in human endothelial microvascular dermal cells (HMVec-D). (**A**) DHE staining in aorta. (**B**) Fluorescence intensity of DHE; staining in aorta shows a restoration in intensity of fluorescence in GK-EA-Aa group in relation to GK-Ctrl. (**C**) DCF staining in aorta. (**D**) Fluorescence intensity of DCF; staining in aorta shows a reduction after the treatment with EA-Aa (n = 5). (**E**) Catalase levels in aorta; treatment with EA-Aa increases catalase levels in GK-EA-Aa (n = 5). Cell viability of HMVec-D cells (n = 3 independent experiments): (**F**) Treatment with EA-Aa for 24 h. (**G**) Previous treatment with EA-Aa (for 30 min) and induction to oxidative stress with H_2O_2 (for 2 h).; a protection of EA-Aa against H_2O_2 is evidenced since 125 µg.mL^{-1}. (**H**) Pre-treatment of cells with NRF2 inhibitor, ML385, followed by incubation with EA-Aa and induction with H_2O_2; EA-Aa increases cell metabolic function in all concentrations and presents differences in relation to the non-inhibited group in 31.25–125 µg.mL^{-1}. * vs. W-Ctrl/Ctrl; # vs. GK-Ctrl/ H_2O_2; & vs. same point without NRF2 inhibitor; *,# $p < 0.05$, ***,###,&&& $p < 0.001$.

4. Discussion

Medicinal plants may be a therapeutic alternative since they have long been applied in the treatment of several diseases, showing important scientifically proven results in improving health condition in several diseases with low side effects and costs [20]. Considering the large consumption of plants and people's adherence to their use as a therapeutic strategy, the relationship between oxidative stress and the development of DM2 and its complications [5,19,21,22], and the relevant antioxidant potential that EA-Aa previously demonstrated [11], we aimed to analyse its potential as a therapeutic strategy in the treatment of DM2 and its complications. The present study provides evidence for the in vitro and in vivo antioxidant potential of EA-Aa, which is associated to the improvement of metabolic profile and vascular function of diabetic rats.

The aqueous extract of *A. aculeata* (EA-Aa) is mainly composed by phenolic compounds and flavonoids, which are probably involved in the therapeutic effects of the extract. Similar data have already been described for the fruit pulp [23] and kernel of *A. aculeata* [24–26]. Plants of the same family, *Arecaceae*, have similar composition as EA-Aa, namely caffeic acid, rutin and quercetin [27–30].

The different therapeutic effects reported here, are in line with previous demonstrations regarding the mechanisms of action of some of the individual compounds of the extract. The more notable effect in the phenotype of the diabetic animals was the reduction of hyperglycaemia, which could be associated to the ferulic (4-hydroxy-3-methoxycinnamic acid), caffeic (3,4-dihydroxycinnamic acid) and gallic (3,4,5-trihydroxybenzoic acid) acids [31]. Ferulic acid seems to reduce glycaemia through the suppression of the activity of the enzyme α-glucosidase and stimulation of insulin secretion [32]. Caffeic acid was associated to higher insulin levels and glucose uptake through AMPK pathway [31,33,34]. Moreover, gallic acid was observed to ameliorate hyperglycaemia and HOMA-IR index [35] and to induce GLUT4 translocation to the plasma membrane [36]. Regarding the flavonoids found in EA-Aa, quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4Hchromen-4-one) was also associated to hypoglycaemic mechanisms [32] such as higher levels of insulin receptor (IR) and insulin receptor substrate (IRS), besides GLUTs and the inhibition of α-glucosidase activity, which are associated to the improvement of insulin resistance [37]. Rutin has been described to increase PPARγ expression and glucose uptake, in addition to also being associated with better insulin sensitivity [38] and the inhibition of α-glucosidase and α-amylase [39]. Similar effects were noted, in particular in the group GK-EA-Aa, namely the decrease in glycemia and the increased levels of AMPK in EAT. Also considering the absence of effects in the ITT (acute insulin action) and the reduction of fasting triglycerides levels, we can consider the mechanism of action more at the level of energy balance stabilization and reduction of oxidative stress, which ends up in a better long-term metabolic function at a systemic level [40–43]. This can be observed in GK-Ctrl, which presented fasting hyperglycaemia and hypertriglyceridaemia. Thus, treatment with EA-Aa reduced triglyceridaemia at baseline levels, which is probably associated with the improvement of AMPK, a known energy sensor and metabolic regulator [44].

Besides the improvement in the metabolic profile of the animals, treatment with EA-Aa also improved the hyperglycaemia-driven endothelial dysfunction. This beneficial effect was previously attributed to the flavonoids rutin [45] and quercetin [46] and the phenolic compounds [47], ferulic [48,49], vanillic, caffeic [46] and gallic acids [50], which may be acting in synergy. An imbalance in the redox state is developed as a consequence of hyperglycaemia, exacerbating ROS production. This effect, together with the reduction of antioxidant defence systems, decreases nitric oxide bioavailability and leads to endothelial dysfunction and vascular damage [19,51]. Thus, considering the reduction in fluorescence probes DHE and DCF, the increased levels of catalase in aorta and the protective effect in HMVec-D cells suggest that the effect of EA-Aa occurs by a stabilization of the redox balance in the organ. It is questionable, however, whether such effects are a consequence of improved glucose metabolism or modulation of antioxidant systems. In fact, an activation of Sirt-1–NRF2 was not detectable in vivo and only a partial dependence was observed in vitro. Moreover, both the reduction of glycaemia and the upregulation of catalase were

observed in diabetic rats, suggesting that EA-Aa may have direct antioxidant effects (as previously demonstrated here), but also modulate such pathways in vivo, which lead us to believe in a long-term effect by EA-Aa.

Several studies have revealed the pharmacological potential of the different phytochemicals that are metabolized by gut microbiota, which present distinct therapeutic effects than the crude extract [52]. Compounds presented in EA-Aa such as gallic, caffeic, ferulic and vanillic acids, rutin and quercetin, all have metabolites produced after in vivo metabolism. Some of them derived from gallic acid include pyrogallol-1-O-glucuronide, 4-OMeGA, 4-OMeGA-3-O-sulfate, pyrogallol-O-sulfate, deoxypyrogallol-O-sulfate, and O-methylpyrogallol-O-sulfate [53]. Ferulic acid is a metabolite of caffeic acid found in the gut lumen [54], which also forms ferulic acid-4-O-glucuronide, ferulic acid-4-O-sulfate, feruloylglycine, and dihydroferulic acid [55], besides being converted into vanillic acid [56]. Moreover, the deglycosylation that happens in the gut lumen forms the quercetin metabolites, quercetin sulfate and quercetin glucoronides [52,57]. Rutin originates the metabolites 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxyphenylacetic acid (DHPAA), 3,4-dihydroxytoluene (DHT), 3-hydroxyphenylacetic acid (HPAA), and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA) [58].

Although the individual contribution of such compounds/metabolites and secondary metabolites is unknown, our results support the effects of EA-Aa at the metabolic level, in improving the glycaemic and lipidic profile and vascular function. These results are probably associated to the mechanistic effect of the compounds present in EA-Aa that may act synergistically, acting to re-establish and maintain the redox balance and consequently prevent complications associated with DM2. In this way, our results support new studies enabling us to understand the mechanisms of phenolic compounds and flavonoids (especially those from EA-Aa) and its metabolites in preventing DM2 complications.

5. Conclusions

Taken together, our results provide evidence for the potential of EA-Aa in improving metabolic pathways in EAT and liver and reducing fasting glycaemia and triglyceride levels. In addition, the treatment with EA-Aa increased vascular redox condition and function, through direct antioxidant properties and modulation of antioxidant systems. Such a reduction of glycaemia and improvement of redox state was associated with improved vascular relaxation in response to acetylcholine, especially in the presence of ascorbic acid. The results obtained in this study suggest that although individual compounds may have a therapeutic role in diabetic complication, their natural combination in plant extracts may also exert beneficial mechanisms. Moreover, the therapeutic effects found here may be distinct if such compounds are administered through non-oral routes and their gut metabolization should be understood in the future. Therefore, the improvement of the metabolic-redox condition by EA-Aa encourages more studies using the compounds present in EA-Aa and their metabolites as a strategy for the development of treatments for the complications associated to DM2.

Author Contributions: Conceptualization: T.M.-A. and P.M. Methodology: Botanical material and isolation of extract: T.M.-A. and K.A. Cell culture and viability assays: T.M.-A.; D.R.-S.; A.S.P.; R.T.; A.M.A.; M.F.B. Animal maintenance and treatment: T.M.-A. *In vivo* procedures and sample collection: T.M.-A.; P.M. Studies of isometric tension of aorta: T.M.-A.; S.O.; S.S. Biochemical analyses: T.M.-A. and P.M. Fluorescence immunocytochemistry and immunohistochemistry: T.M.-A.; S.O.; and P.M. Western Blot: T.M.-A.; A.A.; D.R.-S. and P.M. Statistical analysis: T.M.-A.; S.O. and P.M. Formal analysis: P.M. and K.d.P.S. Investigation: T.M.-A.; P.M. Resources: M.C.-B.; R.S.; P.M. Data curation: P.M. Writing—original draft preparation: T.M.-A. Writing—review and editing: P.M.; M.C.-B.; R.S. and K.d.P.S.; Supervision: P.M. Project administration: P.M. Funding acquisition: P.M. and M.C.-B. All authors have read and agreed to the published version of the manuscript.

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References

1. Ighodaro, O.M. Molecular pathways associated with oxidative stress in diabetes mellitus. *Biomed. Pharmacother.* **2018**, *108*, 656–662. [CrossRef] [PubMed]
2. IDF Diabetes Atlas Worldwide Toll of Diabetes. Available online: https://www.diabetesatlas.org/en/sections/worldwide-toll-of-diabetes.html (accessed on 28 April 2020).
3. Papatheodorou, K.; Banach, M.; Bekiari, E.; Rizzo, M.; Edmonds, M. Complications of Diabetes 2017. Available online: https://www.hindawi.com/journals/jdr/2018/3086167/ (accessed on 28 April 2020).
4. Huang, D.-D.; Shi, G.; Jiang, Y.; Yao, C.; Zhu, C. A review on the potential of resveratrol in prevention and therapy of diabetes and diabetic complications. *Biomed. Pharmacother.* **2020**, *125*, 109767. [CrossRef] [PubMed]
5. Giacco, F.; Brownlee, M. Oxidative stress and diabetic complications. *Circ. Res.* **2010**, *107*, 1058–1070. [CrossRef]
6. Khursheed, R.; Singh, S.K.; Wadhwa, S.; Kapoor, B.; Gulati, M.; Kumar, R.; Ramanunny, A.K.; Awasthi, A.; Dua, K. Treatment strategies against diabetes: Success so far and challenges ahead. *Eur. J. Pharmacol.* **2019**, *862*, 172625. [CrossRef]
7. Figueiredo de Santana Aquino, D.; Monteiro, T.A.; Lima Cardoso, C.A.; Heredia Vieira, S.C.; do Carmo Vieira, M.; de Picoli Souza, K.; Amaya-Farfan, J.; Borges Castro Carvalho, G.C.; Moura, C.S.; Morato, P.N. Investigation of the antioxidant and hypoglycemiant properties of *Alibertia edulis* (L.C. Rich.) A.C. Rich. leaves. *J. Ethnopharmacol.* **2020**, *253*, 112648. [CrossRef]
8. MMA Ministério Do Meio Ambiente—O Bioma Cerrado. Available online: https://www.mma.gov.br/biomas/cerrado (accessed on 9 September 2019).
9. Ribeiro, D.A.; de Oliveira, L.G.S.; de Macêdo, D.G.; de Menezes, I.R.A.; da Costa, J.G.M.; da Silva, M.A.P.; Lacerda, S.R.; de Almeida Souza, M.M. Promising medicinal plants for bioprospection in a Cerrado area of Chapada do Araripe, Northeastern Brazil. *J. Ethnopharmacol.* **2014**, *155*, 1522–1533. [CrossRef] [PubMed]
10. Ribeiro Neto, J.A.; Pimenta Tarôco, B.R.; Batista dos Santos, H.; Thomé, R.G.; Wolfram, E.; de A Ribeiro, R.I.M. Using the plants of Brazilian Cerrado for wound healing: From traditional use to scientific approach. *J. Ethnopharmacol.* **2020**, 112547. [CrossRef] [PubMed]
11. Da Silveira Agostini-Costa, T. Bioactive compounds and health benefits of some palm species traditionally used in Africa and the Americas—A review. *J. Ethnopharmacol.* **2018**, *224*, 202–229. [CrossRef]
12. Silva, P. Caracterização Química e Avaliação Do Potencialantidiabético e Citotóxico de Oleo Extraído de *Acrocomia aculeata* (Macaúba). Master's Thesis, Universidade Federal da Grande Dourados, Dourados, Brazil, 2012.
13. Monteiro-Alfredo, T.; Matafome, P.; Iacia, B.P.; Antunes, K.Á.; dos Santos, J.M.; da Silva Melo da Cunha, J.; Oliveira, S.; Oliveira, A.S.; Campos, J.F.; Magalhães, M.; et al. *Acrocomia aculeata* (Jacq.) Lodd. Ex Mart. Leaves Increase SIRT1 Levels and Improve Stress Resistance. Available online: https://www.hindawi.com/journals/omcl/2020/5238650/ (accessed on 20 March 2020).
14. 3T3-L1 ATCC®CL-173TM Mus Musculus Embryo. Available online: https://www.lgcstandards-atcc.org/products/all/CL-173.aspx?geo_country=ro#culturemethod (accessed on 8 February 2021).
15. Soares, J.; Espadinha, M.; Raimundo, L.; Ramos, H.; Gomes, A.S.; Gomes, S.; Loureiro, J.B.; Inga, A.; Reis, F.; Gomes, C.; et al. DIMP53-1: A novel small-molecule dual inhibitor of p53–MDM2/X interactions with multifunctional p53-dependent anticancer properties. *Mol. Oncol.* **2017**, *11*, 612–627. [CrossRef]
16. EGM-2MV Microvascular Endothelial Cell Growth Medium-2 | Lonza. Available online: https://bioscience.lonza.com/lonza_bs/CH/en/Primary-and-Stem-Cells/p/000000000000185321/EGM--2-MV-Microvascular-Endothelial-Cell-Growth-Medium-2-BulletKit (accessed on 8 February 2021).
17. Rodrigues, T.; Borges, P.; Mar, L.; Marques, D.; Albano, M.; Eickhoff, H.; Carrêlo, C.; Almeida, B.; Pires, S.; Abrantes, M.; et al. GLP-1 improves adipose tissue glyoxalase activity and capillarization improving insulin sensitivity in type 2 diabetes. *Pharmacol. Res.* **2020**, *161*, 105198. [CrossRef]

18. Matafome, P.; Santos-Silva, D.; Crisóstomo, J.; Rodrigues, T.; Rodrigues, L.; Sena, C.M.; Pereira, P.; Seiça, R. Methylglyoxal causes structural and functional alterations in adipose tissue independently of obesity. *Arch. Physiol. Biochem.* **2012**, *118*, 58–68. [CrossRef] [PubMed]
19. Sena, C.M.; Matafome, P.; Louro, T.; Nunes, E.; Seiça, R.M. Effects of atorvastatin and insulin in vascular dysfunction associated with type 2 diabetes. *Physiol. Res.* **2014**, *63*, 189–197. [CrossRef] [PubMed]
20. Shabab, S.; Gholamnezhad, Z.; Mahmoudabady, M. Protective effects of medicinal plant against diabetes induced cardiac disorder: A review. *J. Ethnopharmacol.* **2021**, *265*, 113328. [CrossRef] [PubMed]
21. King, G.L.; Brownlee, M. The cellular and molecular mechanisms of diabetic complications. *Endocrinol. Metab. Clin. North. Am.* **1996**, *25*, 255–270. [CrossRef]
22. Santos, R.X.; Cardoso, S.; Silva, S.; Correia, S.; Carvalho, C.; Crisóstomo, J.; Rodrigues, L.; Amaral, C.; Louro, T.; Matafome, P.; et al. Food deprivation promotes oxidative imbalance in rat brain. *J. Food Sci.* **2009**, *74*, H8–H14. [CrossRef]
23. Da Silva, P.V.B.; Ramiro, M.M.; Iriguchi, E.K.K.; Corrêa, W.A.; Lowe, J.; Cardoso, C.A.L.; Arena, A.C.; Kassuya, C.A.L.; Muzzi, R.M. Antidiabetic, cytotoxic and antioxidant activities of oil extracted from *Acrocomia aculeata* pulp. *Nat. Prod. Res.* **2019**, *33*, 2413–2416. [CrossRef]
24. Nonato, C.D.F.A.; Leite, D.O.D.; Pereira, R.C.; Boligon, A.A.; Ribeiro-Filho, J.; Rodrigues, F.F.G.; da Costa, J.G.M. Chemical analysis and evaluation of antioxidant and antimicrobial activities of fruit fractions of *Mauritia flexuosa* L. f. (Arecaceae). *PeerJ* **2018**, *6*, e5991. [CrossRef] [PubMed]
25. Nunes, Â.A.; Buccini, D.F.; Jaques, J.A.S.; Portugal, L.C.; Guimarães, R.C.A.; Favaro, S.P.; Caldas, R.A.; Carvalho, C.M.E. Effect of *Acrocomia aculeata* kernel oil on adiposity in type 2 diabetic rats. *Plant. Foods Hum. Nutr.* **2018**, *73*, 61–67. [CrossRef]
26. Nunes, Â.A.; Buccini, D.F.; dos Santos Jaques, J.A.; Portugal, L.C.; Guimarães, R.C.A.; Favaro, S.P.; de Araújo Caldas, R.; Carvalho, C.M.E. Effect of dietary *Acrocomia aculeata* kernel oil rich in medium chain fatty acids on type 2 diabetic rats. *J. Funct. Foods* **2020**, *75*, 104295. [CrossRef]
27. Abdelaziz, D.H.A.; Ali, S.A.; Mostafa, M.M.A. Phoenix dactylifera seeds ameliorate early diabetic complications in streptozotocin-induced diabetic rats. *Pharm. Biol.* **2015**, *53*, 792–799. [CrossRef] [PubMed]
28. De Souza, F.G.; de Araújo, F.F.; de Paulo Farias, D.; Zanotto, A.W.; Neri-Numa, I.A.; Pastore, G.M. Brazilian fruits of *Arecaceae* family: An overview of some representatives with promising food, therapeutic and industrial applications. *Food Res. Int.* **2020**, *138*, 109690. [CrossRef]
29. Preetha, P.P.; Girija Devi, V.; Rajamohan, T. Comparative effects of mature coconut water (*Cocos Nucifera*) and glibenclamide on some biochemical parameters in alloxan induced diabetic rats. *Rev. Bras. De Farmacogn.* **2013**, *23*, 481–487. [CrossRef]
30. Renjith, R.S.; Chikku, A.M.; Rajamohan, T. Cytoprotective, antihyperglycemic and phytochemical properties of *Cocos nucifera* (L.) inflorescence. *Asian Pac. J. Trop. Med.* **2013**, *6*, 804–810. [CrossRef]
31. Egawa, T.; Tsuda, S.; Oshima, R.; Goto, A.; Ma, X.; Goto, K.; Hayashi, T. Regulatory mechanism of skeletal muscle glucose transport by phenolic acids. *Phenolic Compd.—Biol. Act.* **2017**. [CrossRef]
32. Chen, L.; Gnanaraj, C.; Arulselvan, P.; El-Seedi, H.; Teng, H. A review on advanced microencapsulation technology to enhance bioavailability of phenolic compounds: Based on its activity in the treatment of type 2 diabetes. *Trends Food Sci. Technol.* **2019**, *85*, 149–162. [CrossRef]
33. Eid, H.M.; Vallerand, D.; Muhammad, A.; Durst, T.; Haddad, P.S.; Martineau, L.C. Structural constraints and the importance of lipophilicity for the mitochondrial uncoupling activity of naturally occurring caffeic acid esters with potential for the treatment of insulin resistance. *Biochem. Pharmacol.* **2010**, *79*, 444–454. [CrossRef]
34. Tsuda, S.; Egawa, T.; Ma, X.; Oshima, R.; Kurogi, E.; Hayashi, T. Coffee polyphenol caffeic acid but not chlorogenic acid increases 5′AMP-activated protein kinase and insulin-independent glucose transport in rat skeletal muscle. *J. Nutr. Biochem.* **2012**, *23*, 1403–1409. [CrossRef]
35. Huang, D.-W.; Chang, W.-C.; Wu, J.S.-B.; Shih, R.-W.; Shen, S.-C. Gallic acid ameliorates hyperglycemia and improves hepatic carbohydrate metabolism in rats fed a high-fructose diet. *Nutr. Res.* **2016**, *36*, 150–160. [CrossRef]
36. Prasad, C.N.V.; Anjana, T.; Banerji, A.; Gopalakrishnapillai, A. Gallic acid induces GLUT4 translocation and glucose uptake activity in 3T3-L1 cells. *FEBS Lett.* **2010**, *584*, 531–536. [CrossRef]
37. Shi, G.-J.; Li, Y.; Cao, Q.-H.; Wu, H.-X.; Tang, X.-Y.; Gao, X.-H.; Yu, J.-Q.; Chen, Z.; Yang, Y. In Vitro and In Vivo evidence that quercetin protects against diabetes and its complications: A systematic review of the literature. *Biomed. Pharmacother.* **2019**, *109*, 1085–1099. [CrossRef]
38. Akuodor, G.; Udia, P.; Bassey, A.; Chilaka, K.; Okezie, O. Antihyperglycemic and antihyperlipidemic properties of aqueous root extract of *Icacina senegalensis* in alloxan induced diabetic rats. *J. Acute Dis.* **2014**, *3*, 99–103. [CrossRef]
39. AL-Ishaq, R.K.; Abotaleb, M.; Kubatka, P.; Kajo, K.; Büsselberg, D. Flavonoids and their anti-diabetic effects: Cellular mechanisms and effects to improve blood sugar levels. *Biomolecules* **2019**, *9*, 430. [CrossRef] [PubMed]
40. Ginsberg, H.N.; Zhang, Y.-L.; Hernandez-Ono, A. Regulation of plasma triglycerides in insulin resistance and diabetes. *Arch. Med. Res.* **2005**, *36*, 232–240. [CrossRef] [PubMed]
41. Koyama, K.; Chen, G.; Lee, Y.; Unger, R.H. Tissue triglycerides, insulin resistance, and insulin production: Implications for hyperinsulinemia of obesity. *Am. J. Physiol. -Endocrinol. Metab.* **1997**, *273*, E708–E713. [CrossRef] [PubMed]
42. Kraegen, E.W.; Cooney, G.J.; Ye, J.; Thompson, A.L. Triglycerides, fatty acids and insulin resistance—Hyperinsulinemia. *Exp. Clin. Endocrinol. Diabetes* **2001**, *109*, 516–526. [CrossRef] [PubMed]

43. Wu, W.; Feng, J.; Jiang, D.; Zhou, X.; Jiang, Q.; Cai, M.; Wang, X.; Shan, T.; Wang, Y. AMPK regulates lipid accumulation in skeletal muscle cells through FTO-dependent demethylation of N 6-methyladenosine. *Sci. Rep.* **2017**, *7*, 41606. [CrossRef]
44. Zong, H.; Ren, J.M.; Young, L.H.; Pypaert, M.; Mu, J.; Birnbaum, M.J.; Shulman, G.I. AMP Kinase Is Required for Mitochondrial Biogenesis in Skeletal Muscle in Response to Chronic Energy Deprivation. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 15983–15987. [CrossRef]
45. Fusi, F.; Saponara, S.; Pessina, F.; Gorelli, B.; Sgaragli, G. Effects of quercetin and rutin on vascular preparations: A comparison between mechanical and electrophysiological phenomena. *Eur. J. Nutr.* **2003**, *42*, 10–17. [CrossRef]
46. Appeldoorn, M.M.; Venema, D.P.; Peters, T.H.F.; Koenen, M.E.; Arts, I.C.W.; Vincken, J.-P.; Gruppen, H.; Keijer, J.; Hollman, P.C.H. Some phenolic compounds increase the nitric oxide level in endothelial cells In Vitro. *J. Agric. Food Chem.* **2009**, *57*, 7693–7699. [CrossRef]
47. Taubert, D.; Berkels, R.; Klaus, W.; Roesen, R. Nitric oxide formation and corresponding relaxation of porcine coronary arteries induced by plant phenols: Essential structural features. *J. Cardiovasc. Pharmacol.* **2002**, *40*, 701–713. [CrossRef]
48. Choi, S.; Il Kim, H.; Hag Park, S.; Jung Lee, M.; Yeoul Jun, J.; Lee Kim, H.; Hoon Chung, J.; Ho Yeum, C. Endothelium-dependent vasodilation by ferulic acid in aorta from chronic renal hypertensive rats. *Kidney Res. Clin. Pract.* **2012**, *31*, 227–233. [CrossRef]
49. Zhou, Z.-Y.; Xu, J.-Q.; Zhao, W.-R.; Chen, X.-L.; Jin, Y.; Tang, N.; Tang, J.-Y. Ferulic acid relaxed rat aortic, small mesenteric and coronary arteries by blocking voltage-gated calcium channel and calcium desensitization via dephosphorylation of ERK1/2 and MYPT1. *Eur. J. Pharmacol.* **2017**, *815*, 26–32. [CrossRef]
50. De Oliveira, L.M.; de Oliveira, T.S.; da Costa, R.M.; de Souza Gil, E.; Costa, E.A.; de Cassia Aleixo Tostes Passaglia, R.; Filgueira, F.P.; Ghedini, P.C. The vasorelaxant effect of gallic acid involves endothelium-dependent and -independent mechanisms. *Vasc. Pharmacol.* **2016**, *81*, 69–74. [CrossRef]
51. Incalza, M.A.; D'Oria, R.; Natalicchio, A.; Perrini, S.; Laviola, L.; Giorgino, F. Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases. *Vasc. Pharmacol.* **2018**, *100*, 1–19. [CrossRef]
52. Traka, M.H.; Mithen, R.F. Plant science and human nutrition: Challenges in assessing health-promoting properties of phytochemicals. *Plant Cell* **2011**, *23*, 2483–2497. [CrossRef]
53. Yang, K.; Zhang, L.; Liao, P.; Xiao, Z.; Zhang, F.; Sindaye, D.; Xin, Z.; Tan, C.; Deng, J.; Yin, Y.; et al. Impact of gallic acid on gut health: Focus on the gut microbiome, immune response, and mechanisms of action. *Front. Immunol.* **2020**, *11*. [CrossRef] [PubMed]
54. Lafay, S.; Morand, C.; Manach, C.; Besson, C.; Scalbert, A. Absorption and metabolism of caffeic acid and chlorogenic acid in the small intestine of rats. *Br. J. Nutr.* **2006**, *96*, 39–46. [CrossRef] [PubMed]
55. Sova, M.; Saso, L. Natural sources, pharmacokinetics, biological activities and health benefits of hydroxycinnamic acids and their metabolites. *Nutrients* **2020**, *12*, 2190. [CrossRef] [PubMed]
56. Topakas, E.; Kalogeris, E.; Kekos, D.; Macris, B.J.; Christakopoulos, P. Bioconversion of ferulic acid into vanillic acid by the thermophilic fungus sporotrichum thermophile. *LWT Food Sci. Technol.* **2003**, *36*, 561–565. [CrossRef]
57. Winterbone, M.S.; Tribolo, S.; Needs, P.W.; Kroon, P.A.; Hughes, D.A. Physiologically relevant metabolites of quercetin have no effect on adhesion molecule or chemokine expression in human vascular smooth muscle cells. *Atherosclerosis* **2009**, *202*, 431–438. [CrossRef]
58. Pashikanti, S.; de Alba, D.R.; Boissonneault, G.A.; Cervantes-Laurean, D. Rutin metabolites: Novel inhibitors of nonoxidative advanced glycation end products. *Free Radic. Biol. Med.* **2010**, *48*, 656–663. [CrossRef] [PubMed]

Paper II

***Acrocomia aculeata* (Jacq.) Lodd. ex Mart. potentiates doxorubicin anticancer activity and attenuates its cardiotoxicity**

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Acrocomia aculeata (Jacq.) Lodd. ex Mart. potentiates doxorubicin anticancer activity and attenuates its cardiotoxicity

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HIGHLIGHTS

- EA-Aa potentiated Dox-induced cytotoxicity in K562 and MCF-7 cells;
- EA-Aa activated mitochondrial death pathways by modulating redox homeostasis;
- EA-Aa protected cardiomyoblast H9c2 against Dox-induced oxidative stress and attenuated ROS generation;
- EA-Aa lacked toxicity and reduced Dox-induced cardiotoxicity in C57Bl/6 mice.

ABSTRACT

Doxorubicin (Dox) displays cardiotoxicity and decreased antioxidant defenses as main side effect on healthy cells. Natural antioxidants might alleviate the side effects observed followed Dox treatment. We evaluated the aqueous extract of *Acrocomia aculeata* leaves (EA-Aa) effects and mechanisms on Dox co-treatment. EA-Aa cytotoxicity was assessed in cancer cells (K562 and MCF-7) besides its ability to potentiate Dox-induced cytotoxicity. K562 cell death and mitochondrial membrane potential were evaluated by flow cytometry. EA-Aa protection against Dox-induced cytotoxicity was assessed by oxidative hemolysis and lipid peroxidation in human red blood cells (RBC) and H9c2 cardiomyoblasts. ML385, an inhibitor of the NRF2 pathway, was used to evaluate NRF2 role in EA-Aa effect. Acute toxicity and cardioprotection of EA-Aa were evaluated in C57Bl/6 mice. The cytotoxic evaluation of EA-Aa showed a relevant decrease in the metabolic activity of cancer cells and potentiated Dox effect. EA-Aa promoted cancer cell death through mitochondrial apoptotic pathways, improved RBC redox state with lower hemolysis and malondialdehyde content, showed no *in vitro* nor *in vivo* toxicity, and antioxidant protection against Dox-induced H9c2 cytotoxicity, partially mediated by NRF2. Lower levels of malondialdehyde were measured in cardiac and renal tissue and nervous system of EA-Aa-treated mice. EA-Aa potentiated Dox anticancer effects with antioxidant and cardioprotective activity, suggesting EA-Aa as a potential Dox pharmacological adjuvant.

KEYWORDS: Antioxidant, chemotherapy side effects, bocaiúva, macaúba, Brazilian cerrado.

ABBREVIATIONS:

Dox, doxorubicin; EA-Aa, aqueous extract of *Acrocomia aculeata*;

1 INTRODUCTION

Doxorubicin (Dox), a potent chemotherapeutic anthracycline [1] extracted from *Streptomyces peuceutius* var. *caesius* in the mid-1960s [2], is used in the treatment of various types of cancer, including breast, lung [1], gastric, ovarian and pancreatic cancer, and hematologic malignancies [2]. Among the mechanisms of Dox anticancer effects, the production of reactive oxygen/nitrogen species (ROS/RNS), apoptosis induction due to cytochrome c release, and DNA damage have been identified [1]. These effects attributed to Dox on cancer cells prompt cytotoxicity of the drug in non-target tissues, namely, in the cardiac tissue. Cardiotoxicity is a recognized side effect associated with Dox therapy [1], which reduces the patient's quality of life and treatment adherence [3]. Besides the increased ROS/RNS production, Dox cardiotoxicity is linked to decreased cardiac antioxidant defenses [4], which leads to a pro-oxidative condition and mitochondrial dysfunction [5].

The nuclear factor erythroid 2-related factor 2 (NRF2) is a transcription factor that regulates several signaling pathways associated with oxidative stress, increasing the transcription level of antioxidant genes and phase II detoxifying enzymes [6]. The reduction of NRF2 levels is associated with more significant Dox-induced cardiotoxicity, in addition to increased cardiac dysfunction [7]. Consequently, compounds that increase NRF2 levels possess a remarkable potential to confer protection against Dox cardiotoxicity [8]. The monitoring of this transcriptional factor is an important target for candidate compounds used in the treatment of Dox-induced cardiotoxicity [9–11]. Among NRF2 activators, phenolic compounds have the potential to improve the clinical side effects associated with Dox chemotherapy [11]. *Acrocomia aculeata* Jacq. (Lodd) ex Mart., a palm native from Brazil, commonly known as macaúba or bocaiúva, has several therapeutic uses described in the literature for its pulp fruit and almond [12]. Recently, our group demonstrated the antioxidant potential of the aqueous extract of *A. aculeata* leaves (EA-Aa), attributing these properties to phenolic compounds and flavonoids [13,14], namely quercetin [15], vanillic [16], ferulic and caffeic acid [17]. These individual compounds have been separately associated with protective effects against Dox-induced toxicity. This study aims to evaluate the *in vitro* and *in vivo* effects of EA-Aa in potentiating Dox anticancer activity while preventing its oxidative stress-associated toxicity in normal cells.

2 MATERIAL AND METHODS

2.1 Plant material and extract preparation

A. aculeata fresh leaves were collected as mentioned before [13] in the region of Grande Dourados, Macaúba district, state of Mato Grosso do Sul (MS) (22°0702.4 S 54°2836.3 W), under the permission of the Brazilian Biodiversity Authorization and Information System (Sistema de Autorização e Informação sobre Biodiversidade, SISBIO; no. 50589). The identification of the species was performed by a plant taxonomist, followed by placing a voucher specimen in the herbarium (DDMS-UFGD) of the Federal University of Grande Dourados, Dourados (MS), Brazil, registration number - 5103. The aqueous extract was prepared as previously described [13].

2.2 Oxidative hemolysis assay

2.2.1 Dox-induced oxidative hemolysis assay

After the approval of the Research Ethics Committee CEP/UFGD nº 5160, peripheral blood was collected from a single adult healthy donor and stored in tubes containing the anticoagulant sodium citrate. A solution of 10% of RBC in physiological solution (NaCl 0.9%) was prepared and previously incubated with EA-Aa in different concentrations (31.25 – 500 $\mu g.mL^{-1}$) at 37 ˚C for 30 min, under constant shaking. Subsequently, the RBC solution was incubated with Dox (300 $\mu g.mL^{-1}$ - IC_{50} hemolysis, diluted in 0.9% NaCl, 4 h, Sigma, USA) to induce damage through oxidative stress. After centrifugation, the optical density of the supernatant was read at 540 nm, and the results were expressed as a percentage of hemolysis based on total hemolysis (incubation of RBC and distilled water). Three independent experiments were performed in duplicate [13].

2.2.2. Evaluation of Dox-induced malondialdehyde (MDA) generation *in vitro*

Following Dox-induced oxidative hemolysis, an aliquot of 10% RBC incubated with Dox and different concentrations of EA-Aa was mixed with 20 nM of thiobarbituric acid (TBA, Merck, Germany), at 96 ˚C for 45 min and then placed in an ice bath for 15 min to stop the reaction. Butanol was added to the tubes to extract the organic fraction of the samples. The absorbance

of the supernatant was determined at 532nm. The concentration of lipid peroxidation product, MDA, was calculated according to equation 1 [13].

$$\text{MDA nmol.mL}^{-1} = \frac{\text{Abs}_{\text{sample}}\ (20\times220.32)}{\text{Abs}_{\text{standard MDA}}} \quad (1)$$

2.3 Cell culture

Human chronic myeloid leukemia (K562) and breast cancer (MCF-7), cultivated in Roswell Park Memorial Institute Medium (RPMI-1640, Sigma, USA) with 10% fetal bovine serum (FBS) and Dulbecco's Modified Eagle's Medium (DMEM, Sigma, USA) 5% FBS, respectively, were used. Normal cells from rat cardiomyoblasts (H9c2) were cultured with DMEM and FBS 10%. Human peripheral mononuclear cells (PBMC) were isolated from a healthy adult donor. For the isolation of PBMC, peripheral blood was collected and mixed with Ficoll-Paque at a 1:1 ratio and centrifuged at 800 x g, 30 min. The PBMC layer was collected and washed twice with phosphate saline buffer (PBS), centrifuged at 330 x g, 10 min, and the cell pellet was resuspended and cultivated in RMPI 20%. All culture media were supplemented with 1% penicillin/streptomycin and cells cultivated at 37 ˚C and 5% CO_2.

2.3.1 Assessment of cell metabolic activity

To evaluate the metabolic activity of the cells after treatment (24 h or 48 h) with EA-Aa (31.25 – 500 µg.mL^{-1}) and with or without Dox (0.5 – 1 µg.mL^{-1}, IC_{20} and IC_{50}, respectively), K562 cells (2 x 10^4 cells.well^{-1}), MCF-7 cells (1 x 10^4 cells.well^{-1}), H9c2 cells (3 x 10^4 cells.well^{-1}) and PBMC cells (1.2 x 10^5 cells.well^{-1}) were seeded in 96-well plates. To determine the role of NRF2 in cardiomyoblast antioxidant protection, the NRF2 inhibitor, ML385 (20 µM), was previously added to H9c2 cells (24 h), followed by EA-Aa (31.25 – 500 µg.mL^{-1}) and Dox (IC_{50} = 20 µg.mL^{-1}) incubations. Cell metabolic activity was determined using the Alamar Blue assay. Suspension cells (K562 and PBMC) were centrifuged at 2000 rpm, 20 min, and the medium was replaced by a solution of RPMI 10% or 20% with 10% of resazurin (0.1 mg.mL^{-1}); Adherent cells (MCF-7 and H9c2) had the medium replaced by the same solution of resazurin in DMEM 5% or 10%. After 24 h of incubation, the absorbance was determined at 570 nm and 600 nm in a BioTek microplate reader (BioTek, Instruments, Inc., Winooski, VT,

USA). The results obtained by the Gen5 program were used to calculate cell metabolic activity, according to equation 2 [13]. Three independent experiments were performed in triplicate.

$$\text{Cellular metabolic activity} = \left(\frac{(Abs_{570}\text{-}Abs_{600})\text{of treated cells}}{(Abs_{570}\text{-}Abs_{600})\text{of control cells}}\right) \times 100 \quad (2)$$

2.3.2. Flow cytometry

2.3.2.1 Cell death

K562 (1 x 10^6 cell.well^{-1}) cell viability was assessed with annexin-V (an-V, Immunostep, Spain) and propidium iodide (PI, Immunostep, Spain). Fluorescein isothiocyanate conjugated an-V and PI were used to label cells, which were analyzed in a four-color FACSCalibur flow cytometer (Becton Dicksson, USA) equipped with a 15 nW argon laser. At least 10^4 events were collected using Cell Quest software (Becton Dickinson, San Jose, CA) and analyzed using Paint-A-Gate Software (Becton Dicksson, USA). Data were expressed in % of viable (an-V-/PI-), apoptotic (an-V+/PI-), late apoptotic/necrotic (an-V+/PI+) and necrotic cells (an-V-/PI+) [18]. Three independent experiments were performed in duplicate.

2.3.2.2. Mitochondrial membrane potential (ΔΨmt)

Mitochondrial membrane potential was performed with the fluorescent probe 5,5,6,6-tetrachloro-1,1,3,3-tetraethyl benzimidazolocar-bocyanine iodide (JC-1, Sigma, USA), mixed with the cells during 15 min, in the dark, at 37 ºC, and assessed by flow cytometry [18]. The JC-1 dye is a lipophilic cationic carbocyanine molecule that naturally exists as monomers and emits green fluorescence. The dye enters the mitochondria, accumulating as reversible complexes named J aggregates, which exhibit red fluorescence. The dye penetrates healthy cells with a normal ΔΨM and accumulates in the functioning organelle and spontaneously forms aggregates with red fluorescence. On the other hand, when in unhealthy or apoptotic cells, where there is a loss of electrochemical potential, JC-1 does not achieve enough mitochondrial concentration to trigger the formation of aggregates, remaining as monomers with green fluorescence [19]. Data are presented as the ratio of aggregates/monomers (A/M), which is

proportional to the mitochondrial membrane potential [18]. Three independent experiments were performed in duplicate.

2.3.3. Intracellular ROS measurement

To determine the effect of EA-Aa on ROS formation in cardiomyoblasts, H9c2 cells were seeded in MilliCells® EZ Slide 8-well glass (Millipore, USA). At confluence, cells were treated with EA-Aa (30 min), followed by the addition of Dox (in the same concentration used in the incubation of cancer cells, 1 $\mu g.mL^{-1}$) overnight. The evaluation of intracellular ROS was carried out with 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA, Invitrogen, USA), following the manufacturer's instructions. DAPI was used to stain the cell nucleus. Images were obtained with a fluorescence microscope (Zeiss Axio Observer Z1) with an incorporated camera (Zeiss, Germany), detected with 504 nm of excitation and 525 nm of emission for DCF and 353 nm of excitation and 465 nm of emission for DAPI (Sigma, USA). The settings were the same for all analyses. The fluorescence quantification was performed in the entire image with the software ImageJ. Three independent experiments were performed in triplicate.

2.4 Animals

2.4.1 Animal maintenance

After the approval of UFGD Ethics Committee on Animal Use nº 10/2017, the experiments were conducted following 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). C57Bl/6 mice were maintained under controlled conditions, 22 ± 2 °C and 12 h light-dark cycle, and were fed *ad libitum*.

2.4.2 EA-Aa acute toxicity determination in C57Bl/6 mice

The acute toxicity test was based on protocols from the Organization for Economic Cooperation and Development (OECD) Guideline 425 [20]. The test was initiated by 8 h fasting, followed by the administration of a single gavage of EA-Aa (2,000 $mg.kg^{-1}$) in a female C57Bl/6 mouse. The animal was frequently observed during the first 24 h. As the first animal did not show any

symptoms of toxicity, the test was continued with the administration of the remaining four mice. The same protocol was repeated with a dose of 5,000 $mg.kg^{-1}$ to define a lethal dose. The control group received only water through gavage (n= 5). After administering the treatment, the animals were observed for 14 days, and during this period, body mass, food, and water intake were measured regularly (Figure 4). The Hippocratic screening was performed to determine behavioral and physiological parameters: urination, defecation, exophthalmos, tremor, catatonia, piloerection, tail erection, hypersalivation, ataxia, lacrimation, pallor/hyperemia/cyanosis of the ears, nose scratching, tail biting, and paw licking. After the study, animals were anesthetized with ketamine/xylazine and euthanized with cervical dislocation followed by organ collection (central nervous system, heart, lungs, liver, spleen, and kidneys), weighing, and macroscopical analysis. Blood was also collected for hematological analysis.

2.4.3. Cardiotoxicity induced by Dox in C57/Bl6 mice

To induce *in vivo* cardiotoxicity with Dox, male C57Bl/6 mice with weight ca. 25 g were randomly distributed between 3 groups (n= 5): 1 - Control (water, p.o.); 2 – Dox (water p.o.); 3 – Dox + EA-Aa (EA-Aa 200 $mg.kg^{-1}$ p.o.). EA-Aa was daily co-administered with Dox (in a cumulative dose of 24 $mg.kg^{-1}$ diluted in 0.9% NaCl), which occurred from the 7^{th} day onwards and on alternate days (according to the protocol presented in Figure 5). The animals were euthanized on the 18^{th} day, with the same protocol described in the acute toxicity assay [21].

2.4.4 Dosage of MDA levels in C57Bl/6 mice organs

The liver, heart, kidney, and nervous system were homogenized in 1.15% potassium chloride (KCl) and centrifuged at 3,000 rpm, 10 min. The supernatant was collected (0.5 mL) and incubated with 1 mL of 10% trichloroacetic acid (TCA) and 1 mL of 20 nM TBA (diluted in 75 nM PBS) at 96 °C/45 min. After samples cooling, 3 mL of butanol was added to the tubes, and the mixture was homogenized, centrifuged (3,000 rpm, 5min), and the absorbance was determined at 532 nm [21].

2.5 Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). All data were compared by One-way ANOVA followed by Student-Newman-Keuls posttest, to compare all means. To evaluate the interactions between cells treated with EA-Aa and Dox, we performed Two-way ANOVA followed by Sidak's posttest. All results were performed with the software GraphPad Prism 7.0. Data were considered significant when $p < 0.05$.

3 RESULTS

3.1 EA-Aa reduces cancer cell metabolic activity and viability, induces mitochondrial dysfunction, and increases Dox-induced cytotoxicity

EA-Aa by itself caused a dose- and time-dependent cytotoxicity in K562 cells, decreasing the metabolic activity of the cells after 48 h (46% to 73%), but not 24h, of treatment (Figure 1 A - B). In MCF-7 cells, EA-Aa cytotoxicity was more evident, with a loss of metabolic activity (37% to 56%) already observed after 24 h of treatment after all tested concentrations (Figure 2 A), and a more robust cytotoxicity effect (52% to 76%) after 48 h treatment (Figure 2 B). Regarding the co-incubation of Dox and EA-Aa, when K562 cells were incubated with Dox, there was a potentiation (~ 10%) of the cytotoxic effect of Dox 0.5 $\mu g.mL^{-1}$ by EA-Aa compared with cells treated with the chemotherapeutic agent alone (Dox 0.5 $\mu g.mL^{-1}$) after 24 h of incubation (Figure 1 C). This effect was time-dependent, since, after 48 h, the reduction in cell metabolic activity was from 14% to 18% compared with cells treated with Dox 0.5 $\mu g.mL^{-1}$ alone (Figure 1 D). The reduction in the metabolic activity of cells treated with EA-Aa and Dox 0.5 $\mu g.mL^{-1}$ after 24 h and 48 h of incubation reached a comparable level to the treatment carried out with twice the concentration (Dox 1 $\mu g.mL^{-1}$). This last condition did not present statistical differences when associated with EA-Aa (Figure 1 E - F). MCF-7 cells presented a similar reduction profile of the metabolic activity of the cells compared to Dox alone, but only for the 48 h time point, with a reduction of 14% to 18% in metabolic activity *vs*. Dox 0.5 $\mu g.mL^{-1}$ (Figure 2 D) and 8% to 12% *vs*. Dox 1 $\mu g.mL^{-1}$ (Figure 2 F).

To evaluate if the decrease in cell survival and proliferation induced by EA-Aa was associated with an increase in cell death, the effect of EA-Aa (250 and 500 $\mu g.mL^{-1}$) alone and in combination with Dox (0.5 and 1 $\mu g.mL^{-1}$) was analyzed by flow cytometry, in K562 cells using double staining with annexin-V and propidium iodide. Figure 3 A shows a lower percentage of alive cells after treatment with EA-Aa (250 and 500 $\mu g.mL^{-1}$). The combination

of EA-Aa with Dox (0.5 and 1 μg.mL^{-1}) decreased cell survival to values similar to those observed in cells treated with Dox 1 μg.mL^{-1} alone (Figure 3 A). Besides, the results show that Dox 1 μg.mL^{-1} acted mostly through necrosis (41.8%), having little effect on initial and late apoptosis (Figure 3 B - E). On the other hand, cells treated with EA-Aa alone presented a large number of cells suffering from apoptosis, while the increase in necrosis was much smaller (Figure 3 B - E). The co-incubation of Dox and EA-Aa significantly reduced Dox-induced necrosis, showing 17% and 15.16% in Dox 0.5 + EA-Aa 250/500 μg.mL^{-1} and 14.5% and 20.5% in Dox 1 + EA-Aa 250/500 μg.mL^{-1} (Figure 3 D). Accordingly, co-incubation significantly increased late apoptosis (Figure 3C).

Regarding the mitochondrial membrane potential measurements, EA-Aa treatment resulted in a decreased ratio of JC-1 aggregates/monomers, which indirectly assesses that parameter. EA-Aa (in both concentrations) by itself showed a reduction in values compared to the control (Figure 3 F). The co-incubation of EA-Aa and Dox presented a more significant reduction in the mitochondrial potential than the cells treated only with Dox or only with EA-Aa, particularly EA-Aa 500 μg.mL^{-1}, potentiating the effect of Dox 1 μg.mL^{-1} (Figure 3 F).

3.2 EA-Aa prevents Dox-induced oxidative hemolysis and oxidative stress in noncancer cells

After confirming EA-Aa cytotoxic potential in cancer cells, we analyzed its possible toxic effects in normal cells, to exclude potential side effects in nontumor cells. The Dox-induced oxidative hemolysis was evaluated in RBC and the generation of MDA, the protective effects of EA-Aa, and the absence of its toxicity are presented in Figure 4 A. After 240 min of incubation, Dox-induced hemolysis in RBC was significantly reduced by treatment with EA-Aa, which showed a 50% protection compared to Dox-treated cells, at the lowest tested concentration. These results are confirmed by the data in Figure 4 B, in which EA-Aa decreased by 30% the Dox-induced increase in MDA levels.

After 24 h of treatment, PBMC reduced cell metabolic activity about 10-20% (Figure 4 C), while H9c2 cardiomyoblasts suffered a similar decrease (Figure 5 A). Therefore, EA-Aa presented also low cytotoxicity in normal cells. Then, we tested the effect of EA-Aa in protecting H9c2 cells from Dox-induced cytotoxicity and oxidative stress (IC_{50} = 20 μg.mL^{-1}). Figure 5 B shows that the previous treatment with EA-Aa restored cell viability, reverting the toxicity showed by the higher dose of Dox (20 μg.mL^{-1}). The NRF2 pathway only partially

mediates this condition since cells incubated with its inhibitor, ML385, had a modest (~ 10%) effect in reducing the protective effect of the extract (Figure 5 B). Accordingly, when cardiomyoblasts (H9c2) were incubated with Dox and EA-Aa under the same conditions of cancer cells (Dox 1 $\mu g.mL^{-1}$), they presented a decrease in DCF fluorescence of ~ 30%, 37.5%, and 52% due to EA-Aa treatment (125, 250 and 500 $\mu g.mL^{-1}$, respectively) in comparison with cells treated with Dox alone (Figure 5 C - D).

3.3 EA-Aa has no acute toxicity and decreases Dox-induced cardiac, nephron, and neurotoxicity in C57Bl/6 mice

Animals treated with EA-Aa 2,000 $mg.kg^{-1}$ and 5,000 $mg.kg^{-1}$ did not present any physiological signs of toxicity, such as significant body weight reduction, physical or behavior changes, or mortality (Figure 6). Only a slight increase in liver weight of 26% in comparison to the control, as presented in Figure 6 H was observed. Hematological changes were observed in the group treated with the higher dose of EA-Aa, with a slight increase in white blood cells and fractions (Table 1). Dox-treated C57Bl/6 mice (Figure 7 A) showed weight loss and lowered caloric intake values compared with the control group, conditions that were not reverted by EA-Aa (Figure 7 B - C). The weight of several organs from the same animals did not change in response to Dox nor EA-Aa treatment, namely, liver, heart, kidney, and central nervous system (Figure 7 D - G). In turn, MDA levels in animals submitted to the chemotherapeutic agent showed an increase of 94% in the heart, which was completely restored after treatment with EA-Aa, with values 23% lower than the control (Figure 7 H). Such protection was also observed in the kidney and brain, with a reduction of 46% and 49% in MDA below baseline levels (Figure 7 I - J).

4 DISCUSSION

In this work, we have observed that *A. aculeata* (Jacq.) Lodd. ex Mart. potentiates Dox anticancer activity and attenuates its cardiotoxicity. Dox is one of the best-described anthracyclines used in chemotherapy due to its wide therapeutic efficacy [5]. However, the side effects have also increased, here untangling cardiotoxicity, the most well-known Dox side effect [22]. Besides the increased levels of pro-apoptotic and pro-inflammatory factors and autophagy markers, Dox-induced mechanisms lead to increased ROS/RNS levels [23] and consequently to oxidative stress. Intending to identify new therapeutic alternatives that could prevent

chemotherapy side effects connected with oxidative stress, medicinal plants with antioxidant properties have become a promising strategy [24–28].

According to Negrette-Guzmán, cancer cells generally produce higher ROS levels than normal cells, a feature that stimulates the tumor in its progression and chemoresistance, involving the upregulation of hypoxia-inducible factor-1 alpha (HIF-1α) and nuclear factor-kappa B (NF-κB) [29]. Chemoresistance also involves the regulation of redox-sensitive transcription factors, such as NRF2, which remains in the cytoplasm when linked to the complex Kelch-like ECH-associated protein 1 (Keap 1) and its dissociation and migration to the nucleus requires the activation of some ROS-mediated kinases (ERK, JNK, p38) or some antioxidants. NRF2 can transcriptionally activate some antioxidant proteins in the nucleus and can up-regulate nuclear respiratory factor 1 (NRF1), which increases mitochondrial function [29]. As we already proved (MONTEIRO-ALFREDO et al., 2020), EA-Aa increases p-NRF2, p-ERK, and catalase in Cos-7 cells, and this suggests that this pathway may be at least in part involved in the antioxidant protection of H9c2 cardiomyoblasts promoted by EA-Aa.

The observed potentiation of Dox-cytotoxic effects and the induction of mitochondrial dysfunction in erythroleukemia cells may be related to the antioxidant and NRF2-activating effects of EA-Aa, already described for other sources of phenolic compounds [6,11,30]. These effects may be related both to the stabilization of radicals in the electron transport chain and to the increase of stress-related protein levels [31]. Assuming that the higher levels of catalase help to stabilize the radicals generated by cancer cells, this effect is probably related to the greater mitochondrial dysfunction in K562 cells, caused by the co-treatment between Dox + EA-Aa, especially at the higher concentrations. When K562 and MCF-7 cells were incubated with EA-Aa and the lower concentration of Dox ($0.5\mu g.mL^{-1}$), the cytotoxic effect was higher than in cells treated with Dox alone ($0.5\mu g.mL^{-1}$) and was similar to the IC_{50} of the cells treated with the higher dose of Dox ($1\mu g.mL^{-1}$). These data show the potentiation of Dox effects by EA-Aa (which occurred mostly through addition) even in the lower concentrations tested, leading to a better chemotherapeutic effect of Dox and significantly lower side effects [32,33]. Regarding the type of death, data from AV/PI double staining, measured by flow cytometry, supports the cell viability assay, showing that Dox effect occurs mainly through necrosis, which may be associated with the mechanism of action of Dox on Topoisomerase II and DNA intercalation, as already described [34]. Instead, EA-Aa induced mitochondrial dysfunction and increased apoptosis, so we must consider EA-Aa as a probable stabilizer of the redox condition.

Among the phytochemical compounds present in medicinal plants, phenolic compounds and flavonoids have shown, in addition to a relevant cytotoxic effect [35], a potential in stabilizing the redox condition present in this type of cells. In a previous study [13], we elucidated the different compounds presented in *A. aculeata* leaves (Supplementary material – Table S1), such as the phenolic compounds gallic, caffeic, vanillic, and ferulic acids, and the flavonoids rutin and quercetin. Besides being responsible for the proven antioxidant effect of EA-Aa, this composition may protect from Dox-induced oxidative hemolysis, MDA formation, and Dox-induced toxicity in H9c2 cells. Indeed, the combination of quercetin and Dox was already shown by Mahbub et al. to activate the mitochondrial apoptotic pathway through caspases 3 and 9 activation [36].

We choose Dox as an oxidative stress inducer for being a source of peroxynitrite ($ONOO^-$), an oxidant agent formed from other two radicals, nitric oxide and superoxide [37], which is a known inducer of oxidative stress-related mechanisms, such as DNA strand breaking, induction of lipid peroxidation, and inhibition of the respiratory chain [38]. Considering this, peroxynitrite produced by Dox is highly related to the development of cardiomyopathy, and its inhibition or reduction may be an alternative for a therapeutic combination. Dox also increased MDA levels in cells [38], and treatment with EA-Aa reduced this oxidative stress biomarker level by 30% in RBC, which was followed by a 50% reduction of the oxidative hemolysis. This protection probably occurs due to the secondary metabolites extracted from *A. aculeata* leaves, as mentioned before. One of the aromatic rings in the flavonoid structure has a hydroxyl configuration that donates hydrogens and electrons to molecules such as peroxyl, peroxynitrite, and hydroxyl, stabilizing them, besides the ion-chelating property of quercetin [39].

Flavonoids are relevant against Dox-induced chronic cardiotoxicity [3] because they prevent both its cytotoxicity and decrease the anticancer effect [40]. To evaluate the potential of EA-Aa against the most noted side effect of Dox chemotherapy, we performed an *in vivo* assay. First, we proved the non-toxicity of EA-Aa in C57Bl/6 mice, and as we expected, the results showed no relevant markers of acute toxicity for 2,000 $mg.kg^{-1}$, proving the safety of consumption. Thus, we next used a ten times lower dose of EA-Aa in the animals for the Dox-induced cardiotoxicity assay. The cardiotoxicity of Dox occurs when administered in cumulative doses [41], and after treatment, the EA-Aa group showed a complete reversion of MDA levels concerning the Dox group, showing the cardioprotective effect of the extract and the reduction of the MDA in the kidney and brain below baseline levels.

5 CONCLUSIONS

Our results demonstrate that EA-Aa shows an attenuating effect on Dox-induced oxidative stress in RBC and H9c2 cardiomyoblast, demonstrated through the decreased cardiotoxicity in C57Bl/6 mice. Besides, EA-Aa did not present toxicity in animals and promoted an additional potentiation of Dox-cytotoxic effect in cancer cells, supporting additional studies to develop a Dox-pharmacological adjuvant based on EA-Aa or its chemical constituents, to reduce Dox side effects.

SUPPLEMENTARY MATERIAL

The phytochemical characterization of the aqueous extract of *A. aculeata* were performed by chromatographic analysis (LC-PDA) in a previous study [13], and identified the following compounds: gallic acid, vanillic acid, caffeic acid, ferulic acid, rutin, and quercetin, as shown in Table S1 (for more information see ref 13).

REFERENCES

[1] Cai Xinyong, Zeng Zhiyi, Hong Lang, Yan Peng, Wu Xiaocheng, Zhang Ping, et al. The role of toll-like receptors in myocardial toxicity induced by doxorubicin. Immunology Letters 2020. https://doi.org/doi.org/10.1016/j.imlet.2019.11.001.

[2] Benjanuwattra J, Siri-Angkul N, Chattipakorn SC, Chattipakorn N. Doxorubicin and its proarrhythmic effects: A comprehensive review of the evidence from experimental and clinical studies. Pharmacological Research 2020;151:104542. https://doi.org/10.1016/j.phrs.2019.104542.

[3] Shabalala S, Muller CJF, Louw J, Johnson R. Polyphenols, autophagy and doxorubicin-induced cardiotoxicity. Life Sciences 2017;180:160–70. https://doi.org/10.1016/j.lfs.2017.05.003.

[4] Alanazi A, Fadda L, Alhusaini A, Ahmad R. Antioxidant, antiapoptotic, and antifibrotic effects of the combination of liposomal resveratrol and carvedilol against doxorubicin-induced cardiomyopathy in rats. J Biochem Mol Toxicol 2020:e22492. https://doi.org/10.1002/jbt.22492.

[5] Wallace KB, Sardão VA, Oliveira PJ. Mitochondrial Determinants of Doxorubicin-Induced Cardiomyopathy. Circ Res 2020;126:926–41. https://doi.org/10.1161/CIRCRESAHA.119.314681.

[6] Sharma A, Parikh M, Shah H, Gandhi T. Modulation of Nrf2 by quercetin in doxorubicin-treated rats. Heliyon 2020;6:e03803. https://doi.org/10.1016/j.heliyon.2020.e03803.

[7] Li S, Wang W, Niu T, Wang H, Li B, Shao L, et al. Nrf2 deficiency exaggerates doxorubicin-induced cardiotoxicity and cardiac dysfunction. Oxid Med Cell Longev 2014;2014:748524. https://doi.org/10.1155/2014/748524.

[8] Tomlinson L, Lu ZQ, Bentley RA, Colley HE, Murdoch C, Webb SD, et al. Attenuation of doxorubicin-induced cardiotoxicity in a human in vitro cardiac model by the induction of the NRF-2 pathway. Biomedicine & Pharmacotherapy 2019;112:108637. https://doi.org/10.1016/j.biopha.2019.108637.

[9] Bai X, Chen Y, Hou X, Huang M, Jin J. Emerging role of NRF2 in chemoresistance by regulating drug-metabolizing enzymes and efflux transporters. Drug Metab Rev 2016;48:541–67. https://doi.org/10.1080/03602532.2016.1197239.

[10] Li B, Kim DS, Yadav RK, Kim HR, Chae HJ. Sulforaphane prevents doxorubicin-induced oxidative stress and cell death in rat H9c2 cells. Int J Mol Med 2015;36:53–64. https://doi.org/10.3892/ijmm.2015.2199.

[11] Han X, Pan J, Ren D, Cheng Y, Fan P, Lou H. Naringenin-7-O-glucoside protects against doxorubicin-induced toxicity in H9c2 cardiomyocytes by induction of endogenous antioxidant enzymes. Food and Chemical Toxicology 2008;46:3140–6. https://doi.org/10.1016/j.fct.2008.06.086.

[12] Agostini-Costa T da S. Bioactive compounds and health benefits of some palm species traditionally used in Africa and the Americas – A review. Journal of Ethnopharmacology 2018;224:202–29. https://doi.org/10.1016/j.jep.2018.05.035.

[13] Monteiro-Alfredo T, Matafome P, Iacia BP, Antunes KÁ, dos Santos JM, da Silva Melo da Cunha J, et al. Acrocomia aculeata (Jacq.) Lodd. ex Mart. Leaves Increase SIRT1 Levels and Improve Stress Resistance. Oxidative Medicine and Cellular Longevity 2020;2020:e5238650. https://doi.org/10.1155/2020/5238650.

[14] Monteiro-Alfredo T, Oliveira S, Amaro A, Rosendo-Silva D, Antunes K, Pires AS, et al. Hypoglycaemic and Antioxidant Properties of Acrocomia aculeata (Jacq.) Lodd Ex Mart. Extract Are Associated with Better Vascular Function of Type 2 Diabetic Rats. Nutrients 2021;13:2856. https://doi.org/10.3390/nu13082856.

[15] Li S, Yuan S, Zhao Q, Wang B, Wang X, Li K. Quercetin enhances chemotherapeutic effect of doxorubicin against human breast cancer cells while reducing toxic side effects of it. Biomedicine & Pharmacotherapy 2018;100:441–7. https://doi.org/10.1016/j.biopha.2018.02.055.

[16] Baniahmad B, Safaeian L, Vaseghi G, Rabbani M, Mohammadi B. Cardioprotective effect of vanillic acid against doxorubicin-induced cardiotoxicity in rat. Res Pharm Sci 2020;15:87–96. https://doi.org/10.4103/1735-5362.278718.

[17] Chegaev K, Riganti C, Rolando B, Lazzarato L, Gazzano E, Guglielmo S, et al. Doxorubicin-antioxidant co-drugs. Bioorg Med Chem Lett 2013;23:5307–10. https://doi.org/10.1016/j.bmcl.2013.07.070.

[18] Pires AS, Marques CR, Encarnação JC, Abrantes AM, Mamede AC, Laranjo M, et al. Ascorbic acid and colon cancer: an oxidative stimulus to cell death depending on cell profile. European Journal of Cell Biology 2016;95:208–18. https://doi.org/10.1016/j.ejcb.2016.04.001.

[19] Sivandzade F, Bhalerao A, Cucullo L. Analysis of the Mitochondrial Membrane Potential Using the Cationic JC-1 Dyeas a Sensitive Fluorescent Probe. Bio Protoc 2019;9. https://doi.org/10.21769/BioProtoc.3128.

[20] Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure | en | OECD n.d. https://www.oecd.org/env/test-no-425-acute-oral-toxicity-up-and-down-procedure-9789264071049-en.htm (accessed January 18, 2021).

[21] dos Santos JM, Alfredo TM, Antunes KÁ, da Cunha J da SM, Costa EMA, Lima ES, et al. Guazuma ulmifolia Lam. Decreases Oxidative Stress in Blood Cells and Prevents Doxorubicin-Induced Cardiotoxicity. Oxidative Medicine and Cellular Longevity 2018;2018:e2935051. https://doi.org/10.1155/2018/2935051.

[22] Monsuez J-J, Charniot J-C, Vignat N, Artigou J-Y. Cardiac side-effects of cancer chemotherapy. International Journal of Cardiology 2010;144:3–15. https://doi.org/10.1016/j.ijcard.2010.03.003.

[23] Yarmohammadi F, Rezaee R, Haye AW, Karimi G. Endoplasmic reticulum stress in doxorubicin-induced cardiotoxicity may be therapeutically targeted by natural and chemical compounds: A review. Pharmacological Research 2021;164:105383. https://doi.org/10.1016/j.phrs.2020.105383.

[24] Casagrande JC, Macorini LFB, Antunes KA, Santos UPD, Campos JF, Dias-Júnior NM, et al. Antioxidant and cytotoxic activity of hydroethanolic extract from Jacaranda decurrens leaves. PLoS One 2014;9:e112748. https://doi.org/10.1371/journal.pone.0112748.

[25] Figueiredo de Santana Aquino D, Monteiro TA, Lima Cardoso CA, Heredia Vieira SC, Vieira M do C, de Picoli Souza K, et al. Investigation of the antioxidant and hypoglycemiant properties of Alibertia edulis (L.C. Rich.) A.C. Rich. leaves. Journal of Ethnopharmacology 2020;253:112648. https://doi.org/10.1016/j.jep.2020.112648.

[26] Lopes RHO, Macorini LFB, Antunes KÁ, Espindola PP de T, Alfredo TM, da Rocha PDS, et al. Antioxidant and Hypolipidemic Activity of the Hydroethanolic Extract of Curatella americana L. Leaves. Oxid Med Cell Longev 2016;2016:9681425. https://doi.org/10.1155/2016/9681425.

[27] Rodrigues AB, de Almeida-Apolonio AA, Alfredo TM, Dantas FG da S, Campos JF, Cardoso CAL, et al. Chemical Composition, Antimicrobial Activity, and Antioxidant Activity of Ocotea minarum (Nees & Mart.) Mez. Oxid Med Cell Longev 2019;2019:5736919. https://doi.org/10.1155/2019/5736919.

[28] Santos UP, Campos JF, Torquato HFV, Paredes-Gamero EJ, Carollo CA, Estevinho LM, et al. Antioxidant, Antimicrobial and Cytotoxic Properties as Well as the Phenolic Content of the Extract from Hancornia speciosa Gomes. PLoS One 2016;11:e0167531. https://doi.org/10.1371/journal.pone.0167531.

[29] Negrette-Guzmán M. Combinations of the antioxidants sulforaphane or curcumin and the conventional antineoplastics cisplatin or doxorubicin as prospects for anticancer chemotherapy. European Journal of Pharmacology 2019;859:172513. https://doi.org/10.1016/j.ejphar.2019.172513.

[30] Giampieri F, Alvarez-Suarez JM, Gasparrini M, Forbes-Hernandez TY, Afrin S, Bompadre S, et al. Strawberry consumption alleviates doxorubicin-induced toxicity by suppressing oxidative stress. Food Chem Toxicol 2016;94:128–37. https://doi.org/10.1016/j.fct.2016.06.003.

[31] Ojha S, Al Taee H, Goyal S, Mahajan UB, Patil CR, Arya DS, et al. Cardioprotective Potentials of Plant-Derived Small Molecules against Doxorubicin Associated Cardiotoxicity. Oxidative Medicine and Cellular Longevity 2016;2016:e5724973. https://doi.org/10.1155/2016/5724973.

[32] Mokhtari RB, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, et al. Combination therapy in combating cancer. Oncotarget 2017;8:38022–43. https://doi.org/10.18632/oncotarget.16723.

[33] Turrini E, Ferruzzi L, Fimognari C. Natural compounds to overcome cancer chemoresistance: toxicological and clinical issues. Expert Opinion on Drug Metabolism & Toxicology 2014;10:1677–90. https://doi.org/10.1517/17425255.2014.972933.

[34] Shin H-J, Kwon H-K, Lee J-H, Gui X, Achek A, Kim J-H, et al. Doxorubicin-induced necrosis is mediated by poly-(ADP-ribose) polymerase 1 (PARP1) but is independent of p53. Scientific Reports 2015;5:15798. https://doi.org/10.1038/srep15798.

[35] de Carvalho JTG, Da Silva Baldivia D, de Castro DTH, dos Santos HF, dos Santos CM, Oliveira AS, et al. The immunoregulatory function of polyphenols: implications in cancer immunity. The Journal of Nutritional Biochemistry 2020;85:108428. https://doi.org/10.1016/j.jnutbio.2020.108428.

[36] Mahbub A, Le Maitre C, Haywood-Small S, Cross N, Jordan-Mahy N. Polyphenols act synergistically with doxorubicin and etoposide in leukaemia cell lines. Cell Death Discov 2015;1:15043. https://doi.org/10.1038/cddiscovery.2015.43.

[37] Ferdinandy P. Peroxynitrite: just an oxidative/nitrosative stressor or a physiological regulator as well? Br J Pharmacol 2006;148:1–3. https://doi.org/10.1038/sj.bjp.0706693.

[38] Lebrecht D, Geist A, Ketelsen U-P, Haberstroh J, Setzer B, Walker UA. Dexrazoxane prevents doxorubicin-induced long-term cardiotoxicity and protects myocardial mitochondria from genetic and functional lesions in rats. British Journal of Pharmacology 2007;151:771–8. https://doi.org/10.1038/sj.bjp.0707294.

[39] Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal 2013;2013:e162750. https://doi.org/10.1155/2013/162750.

[40] Korga A, Józefczyk A, Zgórka G, Homa M, Ostrowska M, Burdan F, et al. Evaluation of the phytochemical composition and protective activities of methanolic extracts of Centaurea borysthenica and Centaurea daghestanica (Lipsky) Wagenitz on cardiomyocytes treated with doxorubicin. Food Nutr Res 2017;61. https://doi.org/10.1080/16546628.2017.1344077.

[41] Mitry MA, Edwards JG. Doxorubicin induced heart failure: Phenotype and molecular mechanisms. Int J Cardiol Heart Vasc 2015;10:17–24. https://doi.org/10.1016/j.ijcha.2015.11.004.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

PM and KP conceived the project and supervised all procedures. Regarding the experimental procedures, red blood cells tests: TM, JM and APAB; cell culture: TM, ASP, IM, and PM; peripheral blood mononuclear cell isolation: TM, ASP, LM and PM; flow cytometry: TM, ASP, IM, ACG and PM; immunocytochemistry: TM and PM; acute *in vivo* studies: TM, JM, KA; *in vivo* cardiotoxicity assay: TM, JM, KA, JC. TM prepared the figures and wrote the manuscript; PJO, PM and KP provided various intellectual support, analyzed the data, reviewed the manuscript and figures; ASP, AMA, MFB, CC, ELS, PM, and KP provided financial support.

Table 1. C57Bl/6 hematological parameters of mice treated with single dose of EA-Aa.

	Control	EA-Aa 2000 mg.kg^{-1}	EA-Aa 5000 mg.kg^{-1}
WBC (10^3.µL^{-1})	2.1 ± 0.3	3.1 ± 0.3*	3.8 ± 0.3**
RBC (10^6.µL^{-1})	10.3 ± 0.4	9.5 ± 0.1	8.0 ± 0.6**
HGB (g.dL^{-1})	14.2± 0.5	13.5 ± 0.1	11.4 ± 0.7**
HCT (%)	56.5 ± 2.5	52.8 ± 0.6	45.5 ± 2.2**
MCV (fL)	55.0 ± 0.5	55.6 ± 0.6	57.6 ± 2.1
MCH (pg)	13.9 ± 0.1	14.2 ± 0.1	14.3 ± 0.3
MCHC (g.dL^{-1})	25.2 ± 0.3	25.7 ± 0.2	25.0 ± 0.3
PLT (10^3.µL^{-1})	367.6 ± 238	408.8 ± 168.7	493.8 ± 202.4
RDW-SD (%)	26.5 ± 0.6	26.9 ± 0.4	28.7 ± 1.7
RDW-CV (%)	19.8 ± 0.4	19 ± 0.2	17.4 ± 0.7**
NEUTROPHIL (10^3.µL^{-1})	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
LYMPHOCYTE (10^3.µL^{-1})	1.9 ± 0.2	3.0 ± 0.3*	3.5 ± 0.2**
MONOCYTE (10^3.µL^{-1})	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1
EOSINOPHYL (10^3.µL^{-1})	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BASOPHIL (10^3.µL^{-1})	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelet; RDW = red cell distribution width. EA-Aa = Aqueous extract of *A. aculeata* leaves. Data are expressed as mean ± SEM. * *vs.* Ctrl, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

FIGURE LEGENDS

Figure 1. EA-Aa reduces cancer cell survival and proliferation, and increases Dox-induced cytotoxicity in Human chronic myeloid leukemia K562 cells. Metabolic activity of EA-Aa-treated (31.25-500µg.mL^{-1}) K562 cells determined by the rezasurin reduction : (A) 24h and (B) 48h. Co-incubation of EA-Aa with Dox 0.5µg.mL^{-1}: (C) 24 h and (D) 48h. Co-incubation of EA-Aa with Dox 1µg.mL^{-1}: (E) 24h and (F) 48h. * *vs*. Ctrl; $^{\#}$ *vs*. Dox 0.5 and 1µg.mL^{-1}; $^{\#\#}$ $p < 0.01$; *** $p < 0.001$.

Figure 2. EA-Aa reduces cancer cell survival and proliferation, besides increasing Dox-cytotoxicity in human breast cancer MCF-7 cells. Metabolic activity of EA-Aa-treated (31.25-500µg.mL^{-1}) MCF-7 determined by the rezasurin reduction: (A) 24h and (B) 48h. Co-incubation between EA-Aa and Dox 0.5µg.mL^{-1}: (C) 24h and (D) 48h. Co-incubation between EA-Aa and Dox 1µg.mL^{-1}: (E) 24h and (F) 48h. * *vs*. Ctrl; $^{\#}$ *vs*. Dox 0.5 and 1µg.mL^{-1}; $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$; *** $p < 0.001$.

Figure 3. EA-Aa reduces mitochondrial membrane potential and induces apoptosis in K562 cells. Flow cytometry analysis by annexin-V and PI staining. Types of cell death: (A) Alive. (B) Initial apoptosis. (C) Late apoptosis/necrosis. (D) Necrosis. (E) Annexin-V and PI staining - summarized data. A (alive); IA (initial apoptosis); LA/N (late apoptosis/necrosis); N (necrosis). (F) JC-1 staining. * *vs*. Ctrl; $^{\#}$ *vs*. Dox 0.5µg.mL^{-1}; $^{\&}$ *vs.* Dox1 µg.mL^{-1}; $^{*,\#}$ $p < 0.05$; $^{**,\#\#,\&\&}$ $p < 0.01$; $^{***,\#\#\#,\&\&\&}$ $p < 0.001$.

Figure 4. EA-Aa shows no toxicity in blood cells while preventing from Dox-induced MDA formation. RBC hemolysis (A) and MDA formation in RBCs exposed to Dox 300µg.mL^{-1} (B). (C) Metabolic activity of PBMC treated with EA-Aa for 24h. * *vs*. Ctrl; $^{\#}$ *vs*. Dox 300/20µg.mL^{-1}. ** $p < 0.01$; $^{***,\#\#\#}$ $p < 0.001$.

Figure 5. EA-Aa prevents cardiomyoblast H9c2 against Dox-induced oxidative stress and reduces ROS generation. Metabolic activity of H9c2 cells treated with EA-Aa (31.25-500µg.mL^{-1}) for 24h (A). Dox-treated (IC_{50} 20µg.mL^{-1}) cells and incubated with NRF2 inhibitor, ML 385 (B). DCF intensity (C) and DCF representative images (DCF, green; DAPI,

blue) (D). * *vs*. Ctrl; # *vs*. Dox 300/20µg.mL^{-1}; & *vs*. respective concentration without NRF2 inhibitor, ML 385. *, #, & $p < 0.05$; ##, && $p < 0.01$; ***, ###, &&& $p < 0.001$.

Figure 6. *In vivo* acute toxicity of EA-Aa in C57Bl/6 mice. (A) Experimental design; (B) Initial and final body mass; (C) Food intake; (D) Brain, (E) Heart, (F) Lung, (G) Kidney, (H) Liver, and (I) Spleen weights. Ctrl group - control mice; 2000 group - treated mice with EA-Aa 2000mg.kg^{-1}; 5000 group - treated mice with EA-Aa 5000mg.kg^{-1}. * *vs*. Ctrl; ** $p < 0.01$.

Figure 7. EA-Aa reduces Dox-induced toxicity in C57Bl/6. (A) Experimental design; (B) Body mass increase; (C) Food intake; and weight of (D) Brain, (E) Heart, (F) Kidney and (G) Liver. MDA levels were determined in (H) Liver, (I) Heart, (J) Kidney and (K) Nervous system. Ctrl group - control mice; Dox group - Dox-induced mice with 24mg.kg^{-1}; EA-Aa - Dox-induced mice treated with EA-Aa 200mg.kg^{-1}. * *vs*. Ctrl; # *vs*. Dox; * $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$.

Figure 1

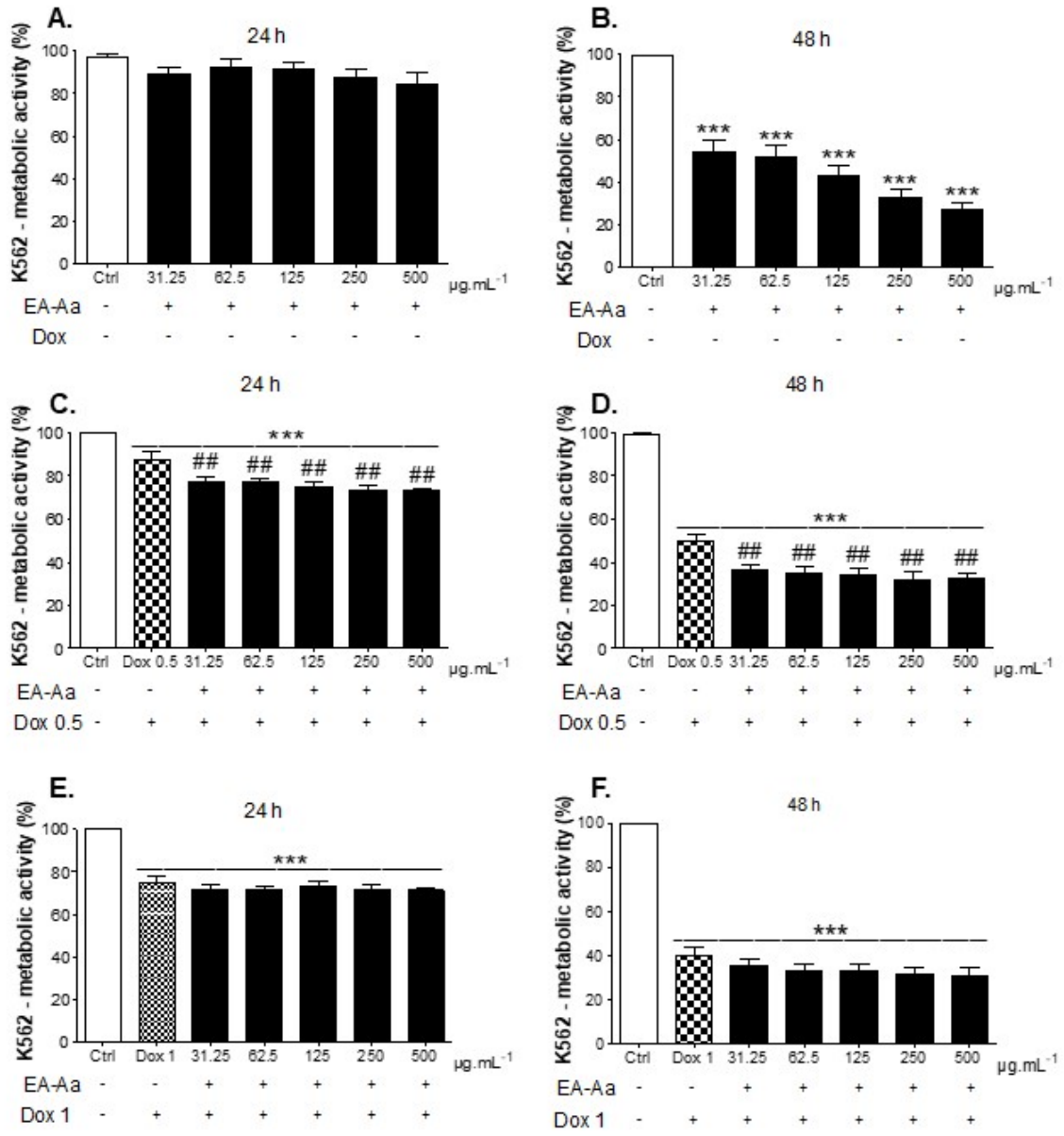

A.
24 h
K562 - metabolic activity (%)
Ctrl 31.25 62.5 125 250 500 µg.mL-1
EA-Aa - + + + + +
Dox - - - - - -
B.
48 h
***
Ctrl 31.25 62.5 125 250 500 µg.mL-1
EA-Aa - + + + + +
Dox - - - - - -
C.
24 h
***
##
Ctrl Dox 0.5 31.25 62.5 125 250 500 µg.mL-1
EA-Aa - - + + + + +
Dox 0.5 - + + + + + +
D.
48 h
***
##
Ctrl Dox 0.5 31.25 62.5 125 250 500 µg.mL-1
EA-Aa - - + + + + +
Dox 0.5 - + + + + + +
E.
24 h
***
Ctrl Dox 1 31.25 62.5 125 250 500 µg.mL-1
EA-Aa - - + + + + +
Dox 1 - + + + + + +
F.
48 h
***
Ctrl Dox 1 31.25 62.5 125 250 500 µg.mL-1
EA-Aa - - + + + + +
Dox 1 - + + + + + +

Figure 2

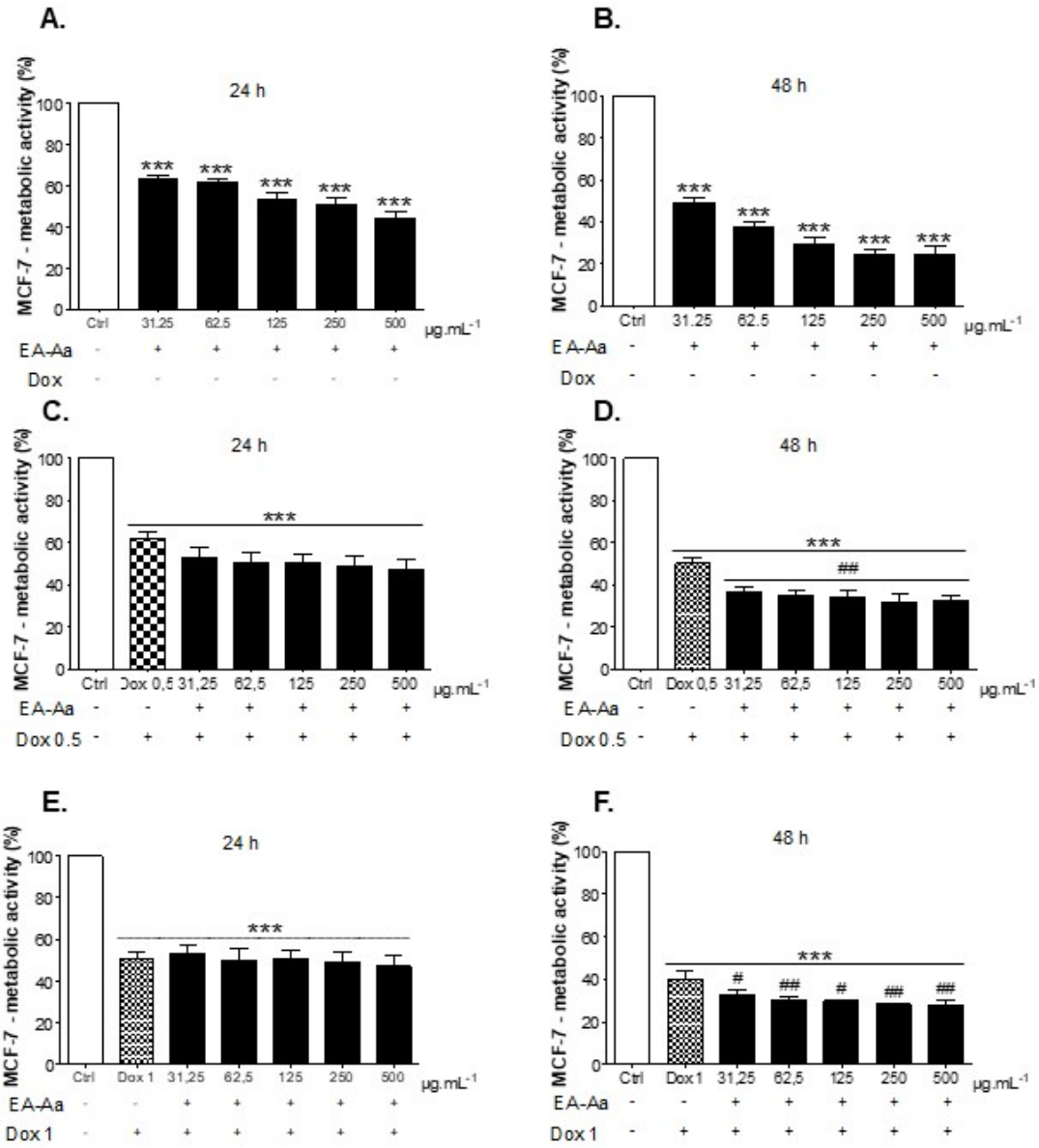
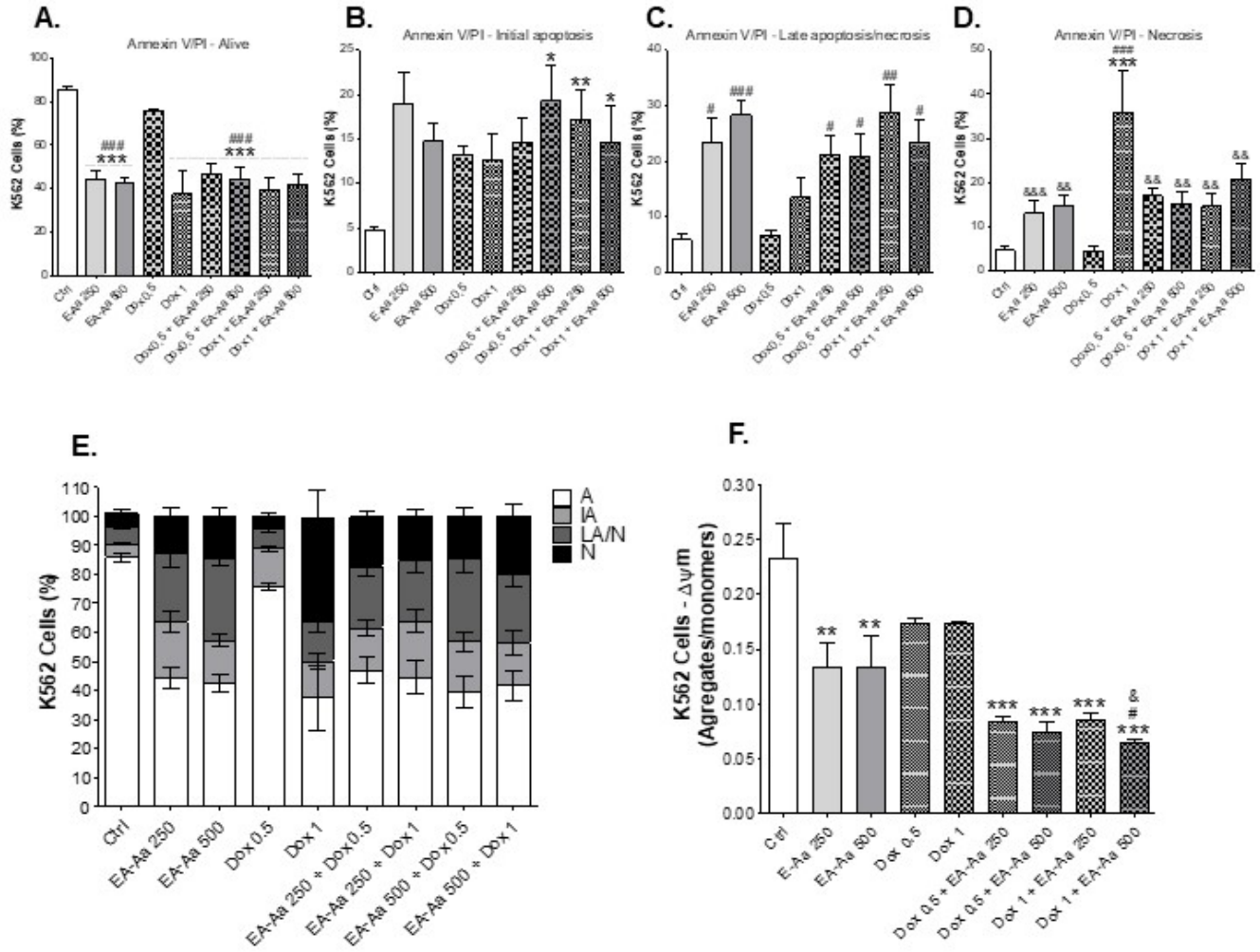

A.
24 h
MCF-7 - metabolic activity (%)
100
80
60
40
20
0
***
***
***
***
***
Ctrl
31.25
62.5
125
250
500
µg.mL-1
EA-Aa - + + + + +
Dox - - - - - -
B.
48 h
MCF-7 - metabolic activity (%)
***
***
***
***
***
Ctrl
31.25
62.5
125
250
500
µg.mL-1
EA-Aa - + + + + +
Dox - - - - - -
C.
24 h
MCF-7 - metabolic activity (%)
***
Ctrl
Dox 0,5
31,25
62,5
125
250
500
µg.mL-1
EA-Aa - - + + + + +
Dox 0.5 - + + + + + +
D.
48 h
MCF-7 - metabolic activity (%)
***
##
Ctrl
Dox 0,5
31,25
62,5
125
250
500
µg.mL-1
EA-Aa - - + + + + +
Dox 0.5 - + + + + + +
E.
24 h
MCF-7 - metabolic activity (%)
***
Ctrl
Dox 1
31,25
62,5
125
250
500
µg.mL-1
EA-Aa - - + + + + +
Dox 1 - + + + + + +
F.
48 h
MCF-7 - metabolic activity (%)
***
#
##
#
##
##
Ctrl
Dox 1
31,25
62,5
125
250
500
µg.mL-1
EA-Aa - - + + + + +
Dox 1 - + + + + + +

Figure 3


A.
Annexin V/PI - Alive
K562 Cells (%)
B.
Annexin V/PI - Initial apoptosis
K562 Cells (%)
C.
Annexin V/PI - Late apoptosis/necrosis
K562 Cells (%)
D.
Annexin V/PI - Necrosis
K562 Cells (%)
Ctrl
E-Aa 250
EA-Aa 500
Dox 0.5
Dox 1
Dox 0.5 + EA-Aa 250
Dox 0.5 + EA-Aa 500
Dox 1 + EA-Aa 250
Dox 1 + EA-Aa 500

E.
K562 Cells (%)
A
IA
LA/N
N
Ctrl
EA-Aa 250
EA-Aa 500
Dox 0.5
Dox 1
EA-Aa 250 + Dox 0.5
EA-Aa 250 + Dox 1
EA-Aa 500 + Dox 0.5
EA-Aa 500 + Dox 1

F.
K562 Cells - Δψm
(Agregates/monomers)
Ctrl
E-Aa 250
EA-Aa 500
Dox 0.5
Dox 1
Dox 0.5 + EA-Aa 250
Dox 0.5 + EA-Aa 500
Dox 1 + EA-Aa 250
Dox 1 + EA-Aa 500

Figure 4

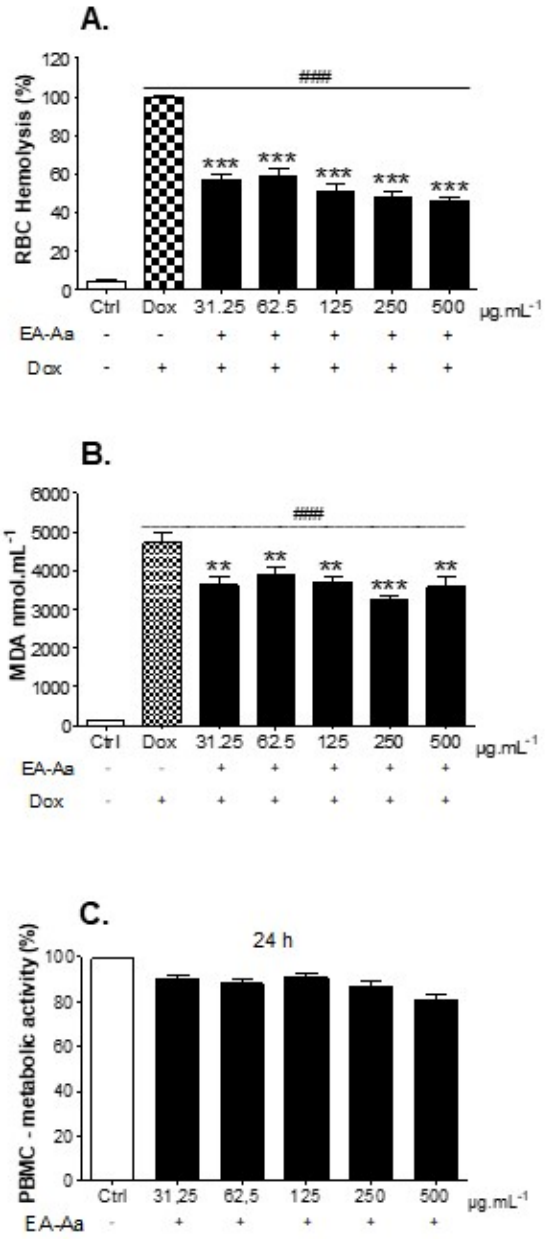

A.
RBC Hemolysis (%)
120
100
80
60
40
20
0
###
***
***
***
***
***
Ctrl
Dox
31.25
62.5
125
250
500
µg.mL-1
EA-Aa - - + + + + +
Dox - + + + + + +
B.
MDA nmol.mL-1
6000
5000
4000
3000
2000
1000
0
####
**
**
**
***
**
Ctrl
Dox
31.25
62.5
125
250
500
µg.mL-1
EA-Aa - - + + + + +
Dox - + + + + + +
C.
24 h
PBMC - metabolic activity (%)
100
80
60
40
20
0
Ctrl
31,25
62,5
125
250
500
µg.mL-1
EA-Aa - + + + + +

Figure 5

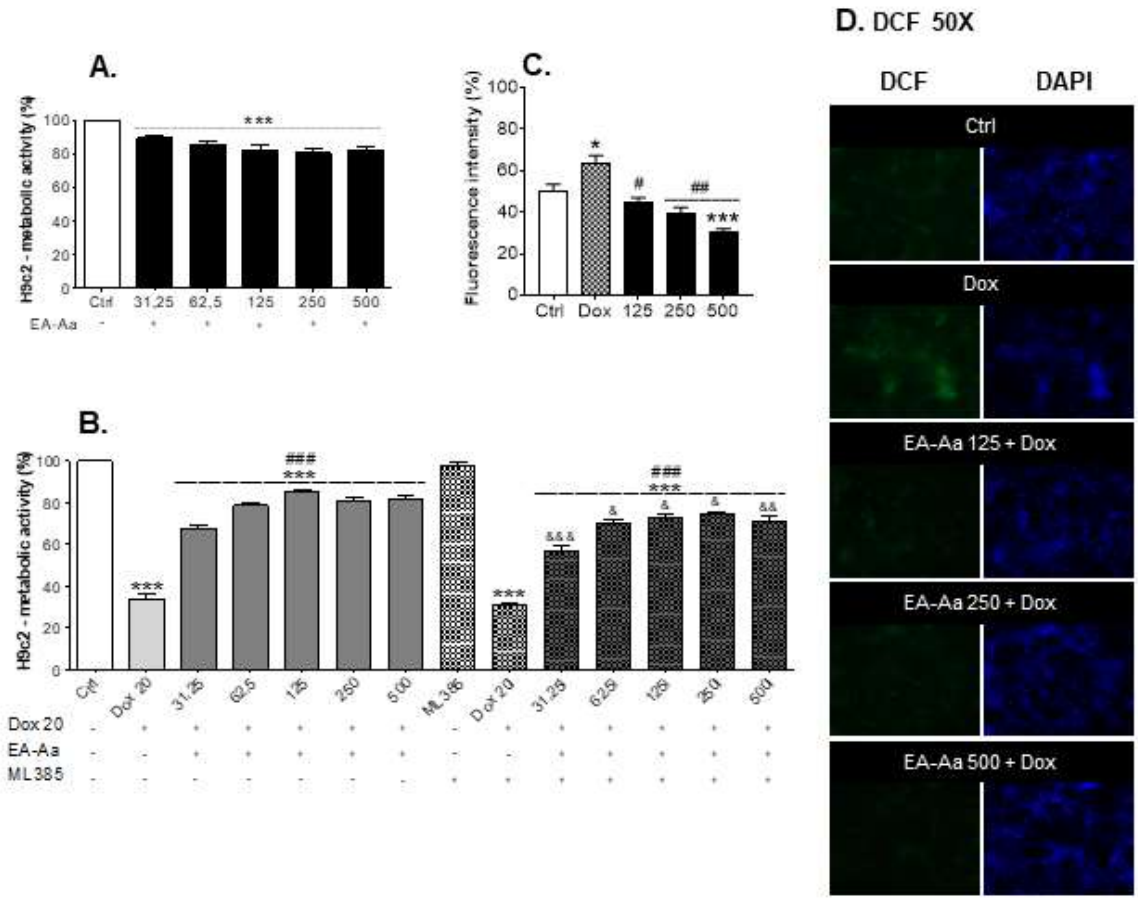

A.
H9c2 - metabolic activity (%)
***
Ctrl 31,25 62,5 125 250 500
EA-Aa
C.
Fluorescence intensity (%)
Ctrl Dox 125 250 500
B.
H9c2 - metabolic activity (%)
Ctrl Dox 20 31,25 62,5 125 250 500 ML385 Dox 20 31,25 62,5 125 250 500
Dox 20
EA-Aa
ML385
D. DCF 50X
DCF
DAPI
Ctrl
Dox
EA-Aa 125 + Dox
EA-Aa 250 + Dox
EA-Aa 500 + Dox

Figure 6

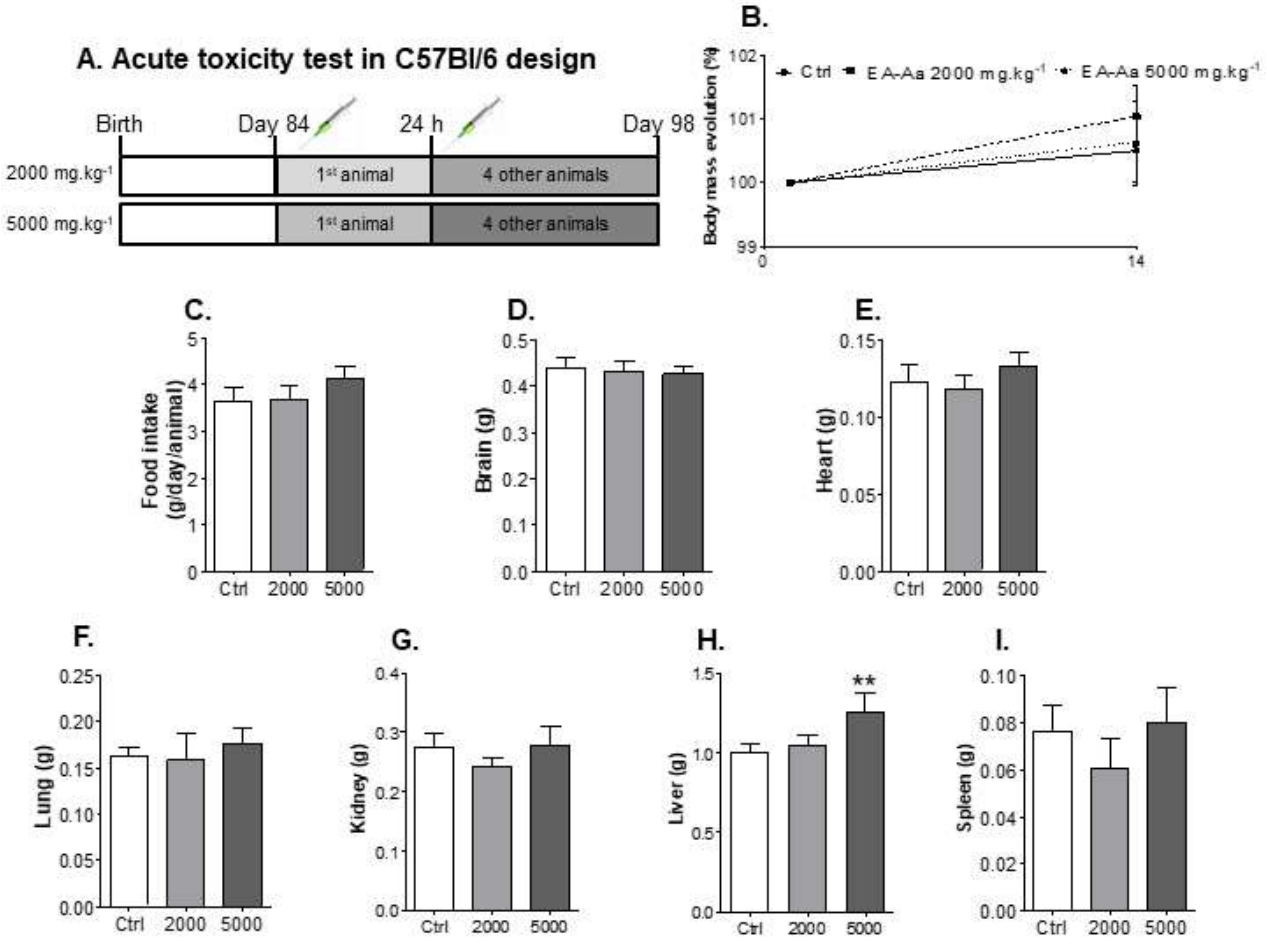
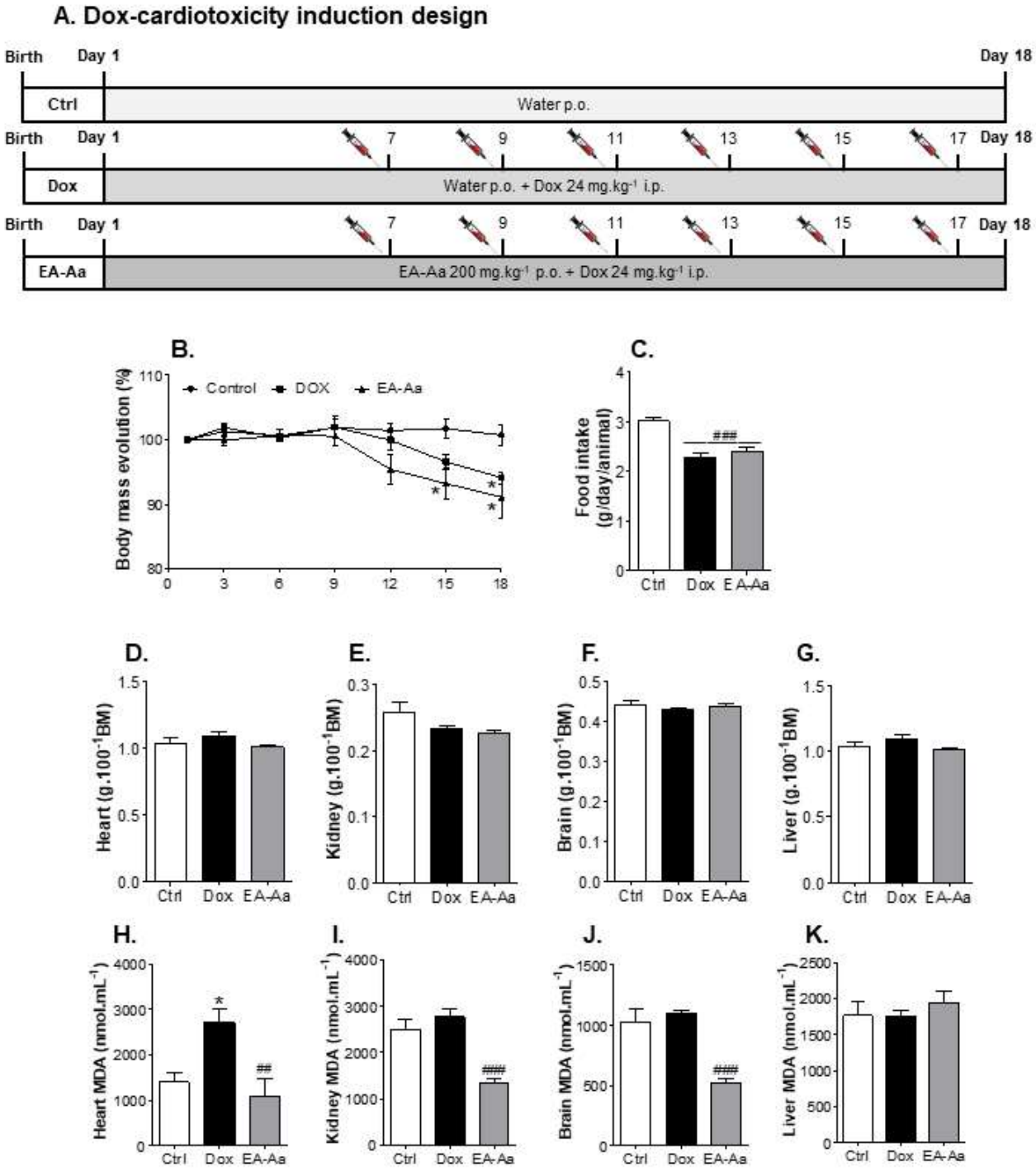

A. Acute toxicity test in C57Bl/6 design
Birth
Day 84
24 h
Day 98
2000 mg.kg-1
5000 mg.kg-1
1st animal
4 other animals
1st animal
4 other animals
B.
Ctrl
EA-Aa 2000 mg.kg-1
EA-Aa 5000 mg.kg-1
Body mass evolution (%)
C.
Food intake (g/day/animal)
D.
Brain (g)
E.
Heart (g)
F.
Lung (g)
G.
Kidney (g)
H.
Liver (g)
**
I.
Spleen (g)
Ctrl
2000
5000

Figure 7


A. Dox-cardiotoxicity induction design
Birth
Day 1
Day 18
Ctrl
Water p.o.
7
9
11
13
15
17
Dox
Water p.o. + Dox 24 mg.kg-1 i.p.
EA-Aa
EA-Aa 200 mg.kg-1 p.o. + Dox 24 mg.kg-1 i.p.
B.
Body mass evolution (%)
Control
DOX
EA-Aa
C.
Food intake (g/day/animal)
###
Ctrl
Dox
EA-Aa
D.
Heart (g.100-1BM)
E.
Kidney (g.100-1BM)
F.
Brain (g.100-1BM)
G.
Liver (g.100-1BM)
H.
Heart MDA (nmol.mL-1)
I.
Kidney MDA (nmol.mL-1)
J.
Brain MDA (nmol.mL-1)
K.
Liver MDA (nmol.mL-1)

SUPPLEMENTARY MATERIAL

Table S1. Chemical composition identified from the LC-PDA analysis of the aqueous extract of *A. aculeata* leaves ($mg.g^{-1}$ ± DP).

Compound	Concentration
Gallic acid	201.6 ± 1.4
Vanillic acid	182.4 ± 0.9
Caffeic acid	124.6 ± 1.2
Ferulic acid	197.9 ± 1.0
Rutin	74.8 ± 0.4
Quercetin	88.7 ± 0.2

6 CONCLUSIONS AND FUTURE PERSPECTIVES

The results of the presented studies proved the therapeutic potential of EA-Aa. Its antioxidant effects revealed in a previous study, which have supported the hypotheses proposed for this thesis, were here on oxidative stress-related diseases: DM2 and its complications and the protective effect against cardiotoxicity induced by chemotherapy performed with Dox.

Taken together, our results evidenced the potential of EA-Aa in improving metabolic pathways in EAT and liver and reducing fasting glycaemia and triglyceride levels in diabetic rats. In addition, treatment with EA-Aa increased vascular redox conditions and function, through direct antioxidant properties and modulation of antioxidant systems. Such a reduction of glycaemia and improvement of redox state were associated with improved vascular relaxation in response to acetylcholine, especially in the presence of ascorbic acid. The results obtained in this study suggest that although individual compounds may have a therapeutic role in diabetic complications, their natural combination in plant extracts may also exert beneficial mechanisms. Moreover, the therapeutic effects found here may be distinct if such compounds are administered through non-oral routes and their gut metabolization should be understood in the future. Therefore, the improvement of the metabolic redox condition by EA-Aa encourages more studies using the compounds present in EA-Aa and their metabolites as a strategy for the development of treatments for the complications associated with DM2.

Additionally, EA-Aa showed cytotoxic activity against cancer cell lines, and in association with Dox, revealed a potentiating effect also on the same cells. Regarding cytotoxicity, EA-Aa caused an increase in mitochondrial dysfunction associated with the predominance of death by apoptosis in tumor cell lines. EA-Aa did not show toxicity *in vitro* in normal cells and *in vivo* in mice. It was able to reduce Dox-induced oxidative stress in erythrocytes, with lower levels of hemolysis and lipid peroxidation, and prevented from Dox-induced cardiotoxicity, showing to be a potential pharmacological adjuvant. Thus, EA-Aa can constitute an important therapeutic strategy for the treatment of both conditions. Future studies could address its role in preventing tumor growth and preventing DOX-induced cardiotoxicity in *in vivo* animal models of tumorigenesis.

ATTACHMENTS

APPROVAL OF THE ETHICS COMITTEE

FUNDAÇÃO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS/UFGD-MS

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Caracterização química e avaliação do potencial farmacológico de Acrocomia aculeata

Pesquisador: Tamaeh Monteiro Alfredo
Área Temática:
Versão: 2
CAAE: 50344215.2.0000.5160
Instituição Proponente: Faculdade de Ciências Biológicas e Ambientais
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.402.556

Apresentação do Projeto:
Trata-se de uma pesquisa envolvendo o estudo dos potenciais terapêuticos e farmacológicos da palmeira bocaiúva (nome científico "Acrocomia aculeata"), nativa do cerrado e encontrada em quantidade considerável na região de Dourados. O objetivo deste estudo é avaliar composição química e o potencial farmacológico das folhas da referida planta. Para isso, as folhas obtidas serão limpas satinizadas, secas, trituradas e submetidas à extração aquosa, etanólica e metanólica. Após o processo serão avaliados compostos fenólicos e flavonóides dos
extratos. A avaliação se dará "in vitro", com sangue coletado de voluntários, será avaliado o potencial antioxidante dos extratos. Os dados gerados poderão subsidiar a elaboração de produtos nutracêuticos e farmacológicos, além de estudos acadêmicos (em nível de iniciação científica, trabalho de conclusão de curso e pós-graduação) que contribuam para a valoração da espécie e conservação da biodiversidade do Centro Oeste.

Objetivo da Pesquisa:
Avaliar a composição química e o potencial farmacológico dos extratos das folhas de Acrocomia aculeata (Jacq.) Lod in vitro. Como objetivos indiretos está a valorização do potencial farmacológico do Cerrado e da diversidade biológica do Centro-Oeste, o que pode fomentar políticas de proteção à esses biomas.

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FUNDAÇÃO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS/UFGD-MS

Continuação do Parecer: 1.402.556

Avaliação dos Riscos e Benefícios:

Os riscos envolvendo seres humanos podem ser classificados como de nível baixo, já que a única exposição humana aos procedimentos é o da extração do sangue que será utilizado para os processos de avaliação. Os riscos estão bem esclarecidos no projeto. Os benefícios são claros e objetivamente apontados.

Comentários e Considerações sobre a Pesquisa:

É uma pesquisa interessante e que acarreta em benefícios diretos e indiretos e com ganhos que vão desde o avanço nos conhecimentos científicos até a preocupação com a proteção ambiental, além de melhorar o bem estar humano.

Considerações sobre os Termos de apresentação obrigatória:

O Termo está claro e contém os itens obrigatórios, deixa claro a possibilidade dos participantes desistirem do processo a qualquer momento, bem como a previsão de que qualquer eventual dano pode ser indenizado. O documento deixa claro que não há ônus para o voluntário.

Recomendações:

Conclusões ou Pendências e Lista de Inadequações:

O projeto se encontra adequado e atende aos requisitos estabelecidos. O parecer é pela aprovação do projeto.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_555379.pdf	12/01/2016 00:54:14		Aceito
Outros	00339.PDF	12/01/2016 00:53:53	Tamaeh Monteiro Alfredo	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	04/01/2016 12:27:27	Tamaeh Monteiro Alfredo	Aceito
Projeto Detalhado / Brochura Investigador	PROJETO.docx	04/01/2016 12:24:18	Tamaeh Monteiro Alfredo	Aceito
Folha de Rosto	CEP.pdf	14/10/2015 12:05:34	Tamaeh Monteiro Alfredo	Aceito

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

FUNDAÇÃO UNIVERSIDADE FEDERAL DA GRANDE DOURADOS/UFGD-MS

Continuação do Parecer: 1.402.556

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

DOURADOS, 03 de Fevereiro de 2016

Assinado por:
Paulo Roberto dos Santos Ferreira
(Coordenador)

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

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, 21 de junho de 2018.

CERTIFICADO

Certificamos que a proposta intitulada ***"Avaliação do Potencial Farmacológico do Extrato Aquoso das Folhas de Acrocomia aculeata (Jacq.) Lodd ex. Mart."***, registrada sob o protocolo de nº 01/2018, sob a responsabilidade de *Kely de Picoli Souza e Tamaeh Monteiro Alfredo* – 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 16/03/2018.

Finalidade	*( ) Ensino (X) Pesquisa Científica*
Vigência da autorização	*30/08/2019 a 30/04/2020*
Espécie/linhagem/raça	*Mus musculus – C57Bl/6*
Nº de animais	60
Peso/idade	50 dias
Sexo	Machos
Origem	Biotério Central UFGD

Melissa Negrão Sepulvida

Melissa Negrão Sepulvida
Coordenadora CEUA

UNIVERSIDADE D COIMBRA FACULDADE DE MEDICINA

iCBR Coimbra Institute for Clinical and Biomedical Research
Faculty of Medicine – University of Coimbra

Órgão Responsável pelo Bem-Estar dos Animais | ORBEA

Instituto de Investigação Clínica e Biomédica de Coimbra (iCBR) | Faculdade de Medicina da Universidade de Coimbra (FMUC)

Título do projeto: Compreensão do impacto precoce e estratégias de redução da glicação como abordagem terapêutica para as complicações da diabetes tipo 2
Investigador responsável: Paulo Nuno Centeio Matafome
Instituição: Faculdade de Medicina da Universidade de Coimbra
Referência interna: 13-2018

PARECER

Na sequência da análise do projeto de investigação envolvendo experimentação animal mencionado em epígrafe, o Órgão Responsável pelo Bem-Estar dos Animais do Biotério do iCBR da FMUC, no cumprimento dos artigos 35º e 43º do Decreto-Lei nº 113/2013 de 7 de Agosto, emite **parecer favorável** à sua realização.

Coimbra, 22 de Março de 2019

Com os melhores cumprimentos,

O ORBEA do iCBR da FMUC,

Flávio Reis (Presidente)

Isabel Vitória Figueiredo | Rosa Fernandes | Francisco Caramelo | Susana Barroso | Graça Melo | Nuno Lima

Instituto de Investigação Clínica e Biomédica de Coimbra (iCBR) | Faculdade de Medicina da Universidade de Coimbra (FMUC) | Azinhaga de Santa Comba, Celas |3000-548 Coimbra | Tel: +351 239 857 700 | Fax: +351 239 857 745 | E-mail: orbea-iCBR@fmed.uc.pt

Responder Excluir Lixo Eletrônico Bloquear ...

Manuscript submitted to Oxidative Medicine and Cellular Longevity

Traduzir a mensagem para: Português (Brasil) | Nunca traduzir do: Inglês

Oxidative Medicine and Cellular Longevity
<mahalakshmi.lakshmanan@hindawi.com>

Qua, 15/09/2021 21:40

Para: Você

Hindawi

Dear Dr. Monteiro-Alfredo,

The manuscript titled "Acrocomia aculeata (Jacq.) Lodd. ex Mart. potentiates doxorubicin anticancer activity and attenuates its cardiotoxicity" has been submitted to Oxidative Medicine and Cellular Longevity by Kely de Picoli Souza.

To confirm the submission and view the status of the manuscript, please verify your details by clicking the link below.

Thank you for submitting your work to Oxidative Medicine and Cellular Longevity.

LOGIN

Kind regards,
Mahalakshmi Lakshmanan
Oxidative Medicine and Cellular Longevity

Responder | Encaminhar