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Avaliação da segurança farmacológica e eficácia de *Plinia cauliflora* (Mart.) Kausel **em coelhos submetidos à um modelo de insuficiência cardíaca induzida por doxorrubicina**

PAULO VITOR MOREIRA ROMÃO

Dourados – MS

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DEDICATÓRIA

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"*Tudo é ousado para quem a nada se atreve"*
(FERNANDO PESSOA)

"*Construí amigos, enfrentei derrotas, venci obstáculos, bati na porta da vida e disse-lhe: Não tenho medo de vivê-la"*
(AUGUSTO CURY)

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATÚRAS E SÍMBOLOS

CDTOX	Cardiotoxicidade
DCRTC	Disfunções cardíacas relacionadas à terapêutica do câncer
DCV	Doença cardiovascular
DOX	Doxorrubicina
DRC	Doença renal crônica
ECG	Eletrocardiograma
ECO	Ecocardiograma/Ecocardiografia
EEPC	Extrato etanólico de *Plinia cauliflora*
EROs	Espécies reativas de oxigênio
FC	Frequência cardíaca
FE	Fração de ejeção
FEVE	Fração de ejeção do ventrículo esquerdo
HAS	Hipertensão arterial sistêmica
HVE	Hipertrofia ventricular esquerda
IAM	Infarto agudo do miocárdio
IC	Insuficiência cardíaca
ICFEI	Insuficiência cardíaca com fração de ejeção intermediária
ICFEP	Insuficiência cardíaca com fração de ejeção preservada
ICFER	Insuficiência cardíaca com fração de ejeção reduzida
OMS	Organização Mundial da Saúde
PA	Pressão arterial
PVC	Pressão vascular central
RMC	Ressonância magnética cardiovascular
SBC	Sociedade Brasileira de Cardiologia
SF	Segurança farmacológica
VE	Ventrículo esquerdo

Avaliação da segurança farmacológica e eficácia de *Plinia cauliflora* (Mart.) Kausel **em coelhos submetidos à um modelo de insuficiência cardíaca induzida por doxorrubicina**

RESUMO

A insuficiência cardíaca (IC) é uma síndrome clínica caracterizada por comprometimento da estrutura e/ou função do coração, promovendo a remodelação cardíaca, bem como distorções no tamanho, forma e função das câmaras cardíacas. Entre os principais fatores de risco para desenvolvimento da IC destacam-se a hipertensão arterial sistêmica e a quimioterapia. Nesse sentido, há um estímulo para a utilização de plantas medicinais no tratamento de doenças cardíacas. A *Plinia cauliflora* (Mart.) Kausel se figura como uma possível alternativa, já que partes da planta são usadas como tratamento natural para asma, diarreia e inflamação e estudos evidenciam que fitoconstituintes como as proantocianidinas e antocianinas protegem especificamente contra doenças cardiovasculares. Desse modo, esse trabalho propõe avaliar a segurança farmacológica e os efeitos cardioprotetores de *P. cauliflora* em um modelo de insuficiência cardíaca induzida por doxorrubicina (DOX) em coelhos. Primeiramente, avaliou-se a segurança farmacológica do extrato de etanólico de *P. cauliflora* (EEPC). Para isso, diferentes grupos de coelhos machos Nova Zelândia foram tratados oralmente com dose única de extrato etanólico de *P. cauliflora* (EEPC) nas doses de 200 e 2000 mg/kg ou veículo (água filtrada). Após esse tratamento, foram observadas durante 24 horas as alterações comportamentais agudas e as possíveis alterações fisiológicas por meio do teste de Irwin modificado, frequência respiratória, frequência cardíaca, pressão arterial, eletrocardiograma e gasometria arterial. Na avaliação da segurança farmacológica não foram observadas alterações comportamentais ou fisiológicas significativas em nenhum dos grupos tratados com EEPC. Ademais, nenhuma das doses de EEPC afetou a frequência respiratória ou a gasometria arterial, e não houve alterações na pressão arterial média ou em parâmetros eletrocardiográficos. Depois desses testes de segurança, foi avaliada a eficácia do EEPC frente um modelo de IC induzida por DOX. Assim, para indução da IC, coelhos fêmeas da linhagem Nova Zelândia receberam administrações semanais de 1,5 mg/kg de DOX pela via intravenosa durante 6 semanas. Concomitantemente, o EEPC foi administrado por via oral em doses de 75 e 150 mg/kg diariamente por 42 dias. O enalapril (5 mg/kg) foi usado como medicamento cardioprotetor de referência e administrado oralmente em doses diárias por 42 dias.

No final do período experimental, a pressão arterial, frequência cardíaca, perfil eletrocardiográfico, reatividade vascular renal, parâmetros séricos, incluindo perfil lipídico, troponina, creatinina, nitrotirosina, malondialdeído, nitrito e peptídeo natriurético cerebral foram mensurados. Além disso, foram realizadas análises histopatológicas das câmaras cardíacas, morfometria ventricular, e investigação do sistema enzimático antioxidante dos tecidos cardíaco e renal. O tratamento com o EEPC induziu importantes respostas, onde alterações hemodinâmicas e funcionais cardíacas foram evitadas e houve prevenção quanto ao remodelamento ventricular. Além disso, o tratamento com o EEPC reduziu a disfunção renal induzida pela IC e modulou positivamente o sistema de defesa antioxidante tecidual.

Palavras-chave: Jabuticaba. Antioxidante. Estresse Oxidativo. Cardiotoxicidade. Quimioterapia.

Pharmacological safety evaluation and efficacy of *Plinia cauliflora* (Mart.) Kausel **in rabbits with a doxorubicin-induced heart failure model**

ABSTRACT

Heart failure (HF) is a clinical disease characterized by impaired heart structure and / or function that promotes cardiac remodeling, such as distortions in the size, shape and function of the cardiac chambers. Among the main risk factors for the development of HF are systemic arterial hypertension and chemotherapy. In this away, In this sense, there is a stimulus for the use of medicinal plants in the treatment of heart disease. *Plinia cauliflora* (Mart.) Kausel is a possible alternative, because parts of the plant are used as a natural treatment for asthma, diarrhea and inflammation and studies show that phytochemicals such as proanthocyanidins and anthocyanins protect specifically against cardiovascular diseases. Thus, this paper proposes to evaluate the pharmacological safety and cardioprotective effects of *P. cauliflora* in a rabbit model of doxorubicin-induced heart failure. First of all, the pharmacological safety of *P. cauliflora* ethanolic extract (EEPC) was evaluated. For that, different groups of male New Zealand rabbits were treated orally with a single dose of ethanolic extract of *P. cauliflora* (EEPC) in the doses of 200 and 2000 mg/kg or vehicle (filtered water). After this treatment, acute behavioral changes and possible physiological changes were observed for 24 hours using the modified Irwin test, respiratory rate, heart rate, blood pressure, electrocardiogram and arterial blood gas analysis. In this pharmacological safety assessment, no significant behavioral or physiological changes were observed in any of the groups treated with EEPC. In addition, none of the EEPC doses affected respiratory rate or arterial blood gases, and there were no changes in mean arterial pressure or electrocardiographic parameters. Following these safety tests, the efficacy of EEPC against a DOX-induced HF model was evaluated. Thus, for HF induction, female rabbits of the New Zealand lineage received weekly administrations of 1.5 mg/kg of DOX intravenously for 6 weeks. Concomitantly, EEPC was administered orally at doses of 75 and 150 mg/kg daily for 42 days. Enalapril (5 mg/kg) was used as a reference cardioprotective medication and administered orally in daily doses for 42 days. At the end of the experimental period, blood pressure, heart rate, electrocardiographic profile, renal vascular reactivity, serum parameters including lipid profile, troponin, creatinine, nitrotirosine, malondialdehyde, nitrite and cerebral natriuretic peptide were evaluated. In addition, cardiac histopathological analyzes, ventricular morphometry and the

antioxidant enzymatic system of the cardiac and renal tissues were investigated. The treatment with EEPC induced important responses where hemodynamic and functional changes were not observed and ventricular remodeling was prevented. In addition, treatment with EEPC reduced HF-induced renal dysfunction and satisfactorily modulated the tissue antioxidant defense system.

Keywords: Jabuticaba. Antioxidant. Oxidative Stress. Cardiotoxicity. Chemotherapy.

SUMÁRIO

1. INTRODUÇÃO

A doença cardiovascular (DCV) é um termo amplo que abrange distúrbios do coração e dos vasos sanguíneos que inclui a hipertensão arterial sistêmica (HAS), doença coronariana, doença cerebrovascular, doença vascular periférica, insuficiência cardíaca (IC) e outras cardiopatias. Nesse aspecto, a IC é uma síndrome clínica caracterizada por comprometimento da estrutura e/ou função do coração, levando a dispneia e fadiga em repouso ou com esforço (KUPPER et al., 2016; WALTHALL et al., 2019). Uma resposta fundamental à lesão do miocárdio ou condições alteradas de débito cardíaco existentes na IC inclui a remodelação do coração, promovendo distorções no tamanho, forma e função das câmaras cardíacas (BURCHFIELD et al., 2013; AZEVEDO et al., 2016). Além disso, é acompanhado por alterações bioquímicas, principalmente no que se refere aos peptídeos natriuréticos, que contribuem de maneira importante para a morbimortalidade dos pacientes (LOURIDAS; LOURIDA, 2012).

Entre os principais fatores de risco para o desenvolvimento da IC se destacam a HAS e a quimioterapia. Estudos epidemiológicos multivariados revelam que a HAS apresenta um alto poder de risco para IC, representando 39% dos casos em homens e 59% em mulheres (MESSERLI et al., 2017). Além disso, em indivíduos com PA >160/90 mm Hg (sistólica e diastólica, respectivamente) esse risco ao longo da vida é o dobro do que àqueles com PA <140/90 mm Hg (WOHLFAHRT; CÍFKOVÁ, 2019). Ademais, a HAS tem sido relatada como a comorbidade mais comumente encontrada em pacientes que apresentam neoplasias, representando cerca de 37% dos casos (FU et al., 2015; ROY et al., 2018). Sendo assim, é necessário entender os efeitos sinérgicos entre a hipertensão e a terapia antineoplásica no desenvolvimento da IC, a curto e longo prazo, para que se alcance uma maior elucidação dos mecanismos fisiopatológios e sobrevida dessa população (KURIAKOSE et al., 2016).

As disfunções cardíacas relacionadas à terapêutica do câncer (DCRTC) têm aumentado entre as mulheres sobreviventes de câncer de mama e, assim, ganhado mais atenção na cardio-oncologia moderna (LARSEN; MULVAGH, 2017). A DCRTC tipo 1 se refere à toxicidade dose-dependente irreversível que resulta de alterações ultraestruturais no miocárdio, sendo tipificado pela cardiotoxicidade das antraciclinas [doxorrubicina (DOX), daunorrubicina, epirrubicina, mitoxantrona e idarrubicina] (EWER; EWER, 2010). Atinente a isso, a DOX é um quimioterápico da classe das antraciclinas usado com sucesso no tratamento de uma ampla gama de cânceres, porém apresenta um alto nível de cardiotoxicidade (CDTOX) que potencializa o desenvolvimento de disfunções cardíacas (MAIA et al. 2017).

Devido à alta prevalência de doenças que acometem o sistema cardiovascular, a Organização Mundial da Saúde (OMS) tem estimulado o desenvolvimento de políticas públicas que priorizam a utilização de plantas medicinais no tratamento dessas doenças e enfatiza a necessidade de novas pesquisas feitas com plantas e outros produtos naturais com a finalidade de aumentar o arsenal terapêutico oferecido à população (MARMITT et al., 2016). Assim, o conhecimento sobre o uso, propriedades farmacológicas e toxicológicas de plantas e produtos derivados é fundamental para garantir sua segurança e eficácia (GROSS et al., 2019).

Diante dessa necessidade científica hodierna, figura a *Plinia cauliflora* (Mart.) Kausel, pertencente a família Myrtaceae, popularmente conhecida como “jabuticaba” ou “jaboticaba”. Essa espécie brasileira é cultivada em todo o país e seu fruto é consumido *in natura* ou em formas processadas, como geleias, sorvetes e licores (ASSIS et al., 2019). Na medicina popular, partes da planta são usadas como tratamento natural para asma, diarreia e inflamação (DONADO-PESTANA et al., 2018). Estes efeitos foram atribuídos aos metabólitos secundários encontrados na fruta. Estudos detectaram compostos fenólicos em suas cascas, como flavonoides, antocianinas e elagitaninos, os quais possuem capacidade antioxidante, anti-inflamatória e atividades quimio-preventivas (WANG et al., 2014; BETTA et al., 2018).

No entanto, as atividades farmacológicas da jabuticaba ainda não estão completamente elucidadas e a função cardioprotetora dessa espécie ainda não foi cientificamente avaliada. Além disso, são escassos os estudos que apontam seus efeitos toxicológicos e farmacodinâmicos. Desse modo, esse trabalho objetiva avaliar a segurança farmacológica e os efeitos cardioprotetores de *P. cauliflora* em um modelo de insuficiência cardíaca induzida por doxorrubicina em coelhos.

2. REVISÃO DE LITERATURA

2.1 Insuficiência cardíaca e suas classificações

A IC é uma síndrome clínica complexa relacionada a um amplo espectro de anormalidades da função ventricular. Esse termo descreve situações onde o coração não tem força suficiente para bombear o sangue para o corpo (PHROMMINTIKUL et al., 2019). Atualmente, é uma das causas mais comuns de hospitalização e morte, com grande impacto nos recursos socioeconômicos (SEVERINO et al., 2019).

De acordo com a Diretriz Brasileira de Insuficiência Cardíaca Aguda e Crônica**,** a principal classificação da IC é determinada a partir da Fração de Ejeção (FE). A FE compara a quantidade de sangue dentro do coração com a quantidade de sangue bombeado para fora durante os movimentos de contração (MELE et al., 2018). A fração ou porcentagem obtida dessa comparação ajuda a descrever o quão bem o coração bombeia sangue para o corpo (KATSI et al., 2017). Em indivíduos saudáveis, a fração de ejeção do ventrículo esquerdo (FEVE) varia de 52% a 72% nos homens, e de 54% a 74% nas mulheres (BLOOM et al., 2017).

Dessa maneira, ao se levar em consideração a FEVE, a IC é classificada em insuficiência cardíaca com fração de ejeção preservada (ICFEP), insuficiência cardíaca com fração de ejeção reduzida (ICFER) e insuficiência cardíaca com fração de ejeção intermediária (ICFEI) (WHITE, 2019). Estudos e registros populacionais no mundo têm sugerido que de 30-75% da população com IC apresentam ICFEP (HARPER et al., 2018). A ICFEP apresenta FEVE $\geq$ 50% e evidência objetiva de disfunção diastólica do VE (SHAH et al., 2017). Essa disfunção diastólica compreende, geralmente, um comprometimento do relaxamento do VE e aumento na rigidez na parede ventricular que contribuem para o aumento da pressão de enchimento do VE (OBOKATA et al., 2019). As causas extra cardíacas de ICFEP são numerosas e incluem etiologias endócrinas como problemas na glândula tireoide, doenças sistêmicas (como sarcoidose e lúpus eritematoso sistêmico), alta ingestão de bebidas alcoólicas e tratamentos quimioterápicos (BLOOM et al., 2017).

Indivíduos com ICFER apresentam FEVE < 40% e disfunção sistólica (MENTZER et al., 2019). A fisiopatologia do ICFER é complexa e geralmente começa com um evento precipitante, como lesão direta no miocárdio ou estado de doença, por exemplo, a doença arterial periférica, infarto do miocárdio e cardiomiopatia dilatada, o que pode levar à redução da contração ventricular (BLOOM et al., 2017).

Além das classificações anteriormente citadas, a partir do ano de 2016 houve o reconhecimento pela Sociedade Europeia de Cardiologia de um novo fenótipo da IC

caracterizada por FEVE entre 40%-49%, que passou a ser denominada de ICFEI (ANDRONIC et al., 2016; MESQUITA et al., 2019). Vários cenários podem levar à ICFEI, como evento isquêmico decorrente de IAM ou transição entre ICFEP e ICFER. Também pode possuir como causa uma ICFER que, após tratamento otimizado, apresenta melhora da FEVE (KAPOOR et al., 2016).

2.1.1 Principais causas

Nas últimas décadas, há um grande esforço para classificar a IC, com a finalidade de melhorar a compreensão dessa síndrome multifacetada e melhor atender às necessidades dos pacientes (MESSERLI et al., 2017). Esse esforço se deve ao quadro clínico da IC ser complexo, pois existem muitas causas possíveis de desenvolvimento e há intrínseca relação com outras doenças **(Figura 1)**.

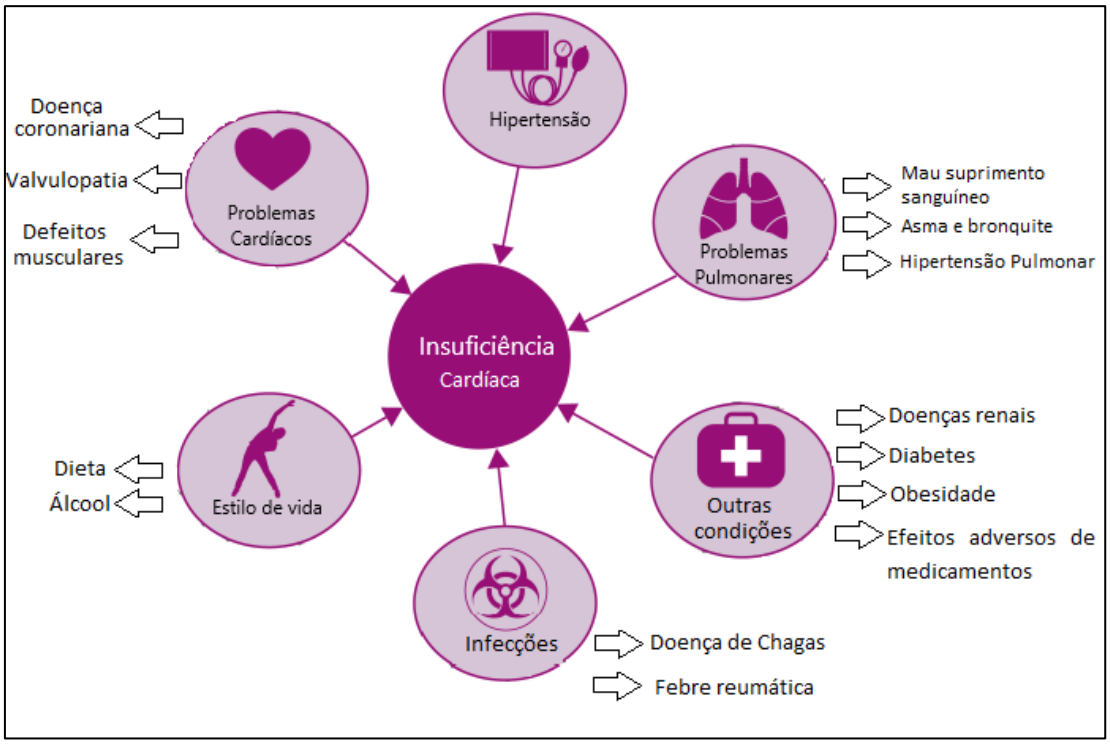

Figura 1: Causas comuns da Insuficiência Cardíaca. (Fonte: Adaptado de Cowie et al., 2014).

Dentro dessa multifatorialidade, a HAS, comumente definida como PA acima de 140/90 mm Hg, é o seu principal fator de risco. Esse risco se deve ao fato de que a HAS provoca remodelamento do VE, um importante mecanismo envolvido na patogênese da IC (PINHO-GOMES; RAHIMI, 2018). Atualmente, está bem estabelecido que a HAS está associada a alterações no tecido vascular e no miocárdico devido a diferentes mecanismos. Entre eles

destaca-se a disfunção endotelial e a constrição arteriolar coronariana, resultando em isquemia, alterações inflamatórias, apoptose e fibrose (FROHLICH, 2009). A remodelação estrutural do coração começa com a hiperplasia dos fibroblastos e hipertrofia vascular da camada muscular lisa, acompanhada pela expansão de colágeno intersticial. As mudanças resultantes na densidade de capilares intramiocárdicos e espessamento arteriolar contribuem para a isquemia nos níveis micro e macrovascular em hipertensos (KANNAN; JANARDHANAN, 2014). No tecido cardíaco esses eventos levam ao estresse físico, resultando na ativação de canais iônicos que regulam positivamente os genes que expressam as células do músculo cardíaco (BERENJI et al., 2005).

Também é importante salientar que a IC está associada ao alto risco de disfunção renal e ao desenvolvimento de doença renal crônica (DRC), uma vez que a insuficiência renal prediz a disfunção do VE (AL-NAHER et al., 2018). Isso significa que o início da DRC é um fator de risco para o desenvolvimento subsequente de IC (HERZOG et al., 2011). Ademais, as alterações na perfusão renal são responsáveis por grande parte do comprometimento renal em pacientes com IC (SCHROTEN et al., 2016). Um mecanismo proposto dessa ligação se refere ao fato da IC causar aumento da pressão venosa central (PVC), que ocorre a medida que os ventrículos se dilatam e o débito cardíaco diminui. Essa contrapressão venosa pode ser transmitida à vasculatura renal, causando congestão venosa renal crônica, o que reduz o fluxo sanguíneo glomerular e, consequentemente, diminui o gradiente de pressão entre arteríolas aferentes e eferentes (SEGALL et al., 2014).

2.1.2 IC resultante do tratamento do câncer

O sucesso no diagnóstico e tratamento de muitas neoplasias resultou no crescimento exponencial do número de pessoas que vivem curadas do câncer ou que o tratam como uma doença crônica. Entretanto, esse êxito da medicina moderna cria uma nova realidade, pois os sobreviventes desenvolvem DCV resultantes dos tratamentos disponíveis (ZAMORANO et al., 2016; BERTERO et al., 2018).

Terapias contra o câncer, incluindo quimioterapia citotóxica, terapias moleculares direcionadas e radioterapia têm sido associadas ao dano miocitário, disfunção ventricular esquerda, IC, trombogênese, HAS e isquemia (SORRENTINO et al, 2012; BLOOM et al. 2017). A IC como resultado da terapia do câncer, tem sido associada a um aumento de 3,5 vezes no risco de mortalidade em comparação à cardiomiopatia idiopática (GUHA et al., 2019).

A radioterapia, por exemplo, pode causar toxicidade direta ao coração levando à IC, tanto por dano miocárdico direto quanto indiretamente, através de um IAM promovido por doença arterial coronariana induzida pela radiação (SAIKI et al., 2017). Já a quimioterapia, como nos casos onde se utilizam quimioterápicos da classe das antraciclinas, pode ocorrer comprometimento funcional da parede miocárdica ventricular que, com o aumento das doses, pode levar a irreversibilidade do dano por necrose miocítica, apoptose e fibrose (MCGOWAN et al., 2017). Dessa forma, quando comparado com outras modalidades, a IC induzida pela terapia antineoplásica é considerada uma das formas mais graves devido à piora de prognóstico (CHATTERJEE et al., 2010).

2.1.2.1 Doxorrubicina

A doxorrubicina (DOX) é um quimioterápico da classe das antraciclinas usado com sucesso para o tratamento de tumores (OJHA et al., 2016). Descoberta no final da década de 60 representou um dos grandes avanços na luta contra o câncer e continua sendo um dos antineoplásicos mais utilizados, tanto individualmente quanto em associação com outros quimioterápicos (ARCAMONE et al., 1978; CARVALHO et al., 2009).

É uma droga citotóxica potente isolada a partir de colônias de bactérias *Streptomyces peucetius* que faz parte de vários regimes terapêuticos para o controle de neoplasias, incluindo cânceres de mama, pulmão, gástrico, ovariano e linfoma Hodgkin e não-Hodgkin (THORN et al., 2011). Entretanto, seu uso clínico é limitado pela toxicidade sistêmica e, principalmente, pela indução de toxicidade cardíaca cumulativa e dose-dependente (LEE et al., 2017).

A CDTOX é um dos eventos adversos mais graves e de grande importância na sobrevida de pacientes submetidos ao tratamento com antraciclinas, podendo desenvolver-se de forma aguda ou crônica (CAI et al., 2019). Muitos fatores de risco podem predispor um paciente a CDTOX induzida por quimioterápicos, incluindo a dose cumulativa administrada, via e frequência com que são realizadas as infusões, histórico de problema cardiovascular pré-existente, dentre outros (KIBUDDE et al., 2019).

Diversos mecanismos celulares têm sido propostos para explicar a CDTOX induzida pela DOX, contudo o mecanismo exato e suas consequências metabólicas permanecem incertos. Entre os mecanismos responsáveis pela patogênese da agressão ao miocárdio promovido pela DOX destacam-se o estresse oxidativo e a disfunção mitocondrial (GORINI et al., 2018; SONGBO et al., 2019). Por muito tempo, predominou-se a importância da necrose como mecanismo responsável pela morte celular na cardiomiopatia induzida pela DOX, todavia um crescente número de evidências tem reconhecido a apoptose como mecanismo fundamental

para a morte das células cardíacas diante da terapia antraciclínica (NEDELJKOVIC et al., 2019; RENU et al., 2019). Ademais, evidências bioquímicas e fisiológicas sugerem que a administração de DOX seja responsável pela formação de radicais livres, que estimulam a peroxidação lipídica, alteram a integridade da membrana celular e causa alterações no transporte de cálcio mitocondrial, resultando na morte de cardiomiócitos (RENU et al., 2018; KOLEINI et al., 2019).

2.2 Papel do estresse oxidativo na IC

Os radicais livres são átomos ou moléculas altamente reativos que possuem em sua camada externa um ou mais elétrons desemparelhados e são formados quando o oxigênio interage com certas moléculas (LIGUORI et al., 2018). Esses radicais podem ser produzidos nas células, perdendo ou aceitando um elétron que se comportam como oxidante ou redutor (PHANIENDRA et al., 2015).

O estresse oxidativo é definido como uma desregulação entre a produção de espécies reativas de oxigênio (EROs) e os mecanismos endógenos de defesa antioxidante, o chamado "estado redox" (VAN DER POL et al., 2019). Quando em concentração baixa as EROs desempenham função de manutenção da homeostase celular, porém o seu excesso provoca disfunções celulares e proteicas, peroxidação lipídica, dano ao DNA e, eventualmente, leva a irreversibilidade de danos celulares (MONDAL et al., 2013). Dessa forma, pesquisa-se continuamente o potencial do dano oxidativo em DCV (PANTH et al., 2016).

No coração, o excesso de EROs leva ao desenvolvimento e progressão da remodelação miocárdica e prejudica diretamente a eletrofisiologia dos cardiomiócitos, modificando as proteínas centrais de acoplamento, os canais de cálcio, sódio e potássio (TSUTSUI et al., 2011; MUNZEL et al., 2017). Além disso, altera-se a atividade do retículo sarcoplasmático reduzindo a sensibilidade do cálcio nos miofilamentos. Por fim, as EROs possuem capacidade pró-fibrótica, induzindo a proliferação de fibroblastos cardíacos e metaloproteinases, resultando em remodelamento extracelular (SENONER; DICHTL, 2019). Em conjunto, essas alterações fisiológicas são as responsáveis pela diminuição da capacidade de bombeamento sanguíneo inerente à IC.

2.3 Diagnóstico e tratamento

Atualmente, o diagnóstico da IC é feito por meio de investigações como exames físicos, bioquímicos e imagiológicos, sendo utilizados para confirmar o diagnóstico, descartar outras

causas, determinar a classificação da IC e investigar possíveis etiologias (ARIYACHAIPANICH et al., 2019). Os parâmetros físicos são definidos como métodos de exame não invasivos e não eletrônicos, incluindo estetoscópio e esfigmomanômetro. Tais parâmetros incluem os sinais vitais, como PA e frequência cardíaca (FC) nas posições reclinada e ereta, frequência respiratória, auscultação do tórax e extensão do fígado (LEIER; CHATTERJEE, 2007).

O eletrocardiograma (ECG) em repouso é uma investigação não invasiva recomendada na avaliação inicial dos pacientes com IC. Isso ocorre porque o ECG é crucial para a detecção de muitas anormalidades que podem causar ou piorar a IC (KARAYE et al., 2008; SEFEROVIC et al., 2019). Seu uso é importante para avaliar sinais de cardiopatia estrutural, como a HVE, isquemia miocárdica, áreas de fibrose, distúrbios da condução atrioventricular, bradicardia ou taquiarritmias, que podem demandar cuidados e tratamentos específicos (SBC, 2018). Além disso, exames de imagem como ecocardiograma (ECO) e ressonância magnética cardiovascular (RMC) são úteis nessa avaliação.

Historicamente, o uso do ECO começou com o modo M (multidirecional), técnica que continua sendo útil para avaliação das dimensões da parede e das câmaras cardíacas. O avanço promovido pela ECO bidimensional (2D) adicionou a capacidade de avaliar o volume do VE e a presença de doença valvular. Com a progressão da ECO tridimensional (3D) houve melhora na precisão e confiabilidade com as quais o volume e a função das câmaras são medidos, bem como a avaliação da regurgitação mitral. A avaliação ecocardiográfica por Doppler tornou-se um coadjuvante indispensável no diagnóstico da IC, uma vez que essa técnica permitiu avaliação da artéria pulmonar, determinação da pressão atrial direita, a capacidade de enchimento do VE e lesões valvares regurgitantes (MARWICK, 2015). Já no que tange à utilização da RMC, ela desempenha um papel complementar, pois informa o prognóstico e orienta a tomada de decisões principalmente quando o ECO não fornece resultados conclusivos (PETERZAN et al., 2016).

As análises bioquímicas também são importantes para a avaliação da IC, incluindo o hemograma, urinálise, perfil metabólico completo para os níveis de eletrólitos séricos como cálcio e magnésio, dosagem de ureia, creatinina, perfil lipídico e marcadores específicos da função cardíaca como o peptídeo natriurético cerebral (BNP) (INAMDAR et al., 2016). O BNP possui sensibilidade de 70% e especificidade de 99% e é um neuro-hormônio secretado pelos ventrículos e, em menor grau, nos átrios. Em resposta à expansão e pressão de volume, o precursor proBNP é secretado nos ventrículos e se decompõe em suas duas formas clivadas, o fragmento N-terminal biologicamente inerte (NT-proBNP) e hormônio biologicamente ativo

(BNP). Seu nível é um forte preditor de risco de morte e eventos cardiovasculares em pacientes previamente diagnosticados com IC ou disfunção cardíaca (INAMDAR et al., 2016; CAO et al., 2019).

O tratamento da IC é baseado em medidas farmacológicas e não-farmacológicas. A primeira inclui inibidores da enzima conversora de angiotensina, betabloqueadores, diuréticos, bloqueadores do canal de cálcio e drogas antiarrítmicas. Já as medidas não-farmacológicas incluem mudanças na alimentação, restrição da ingestão de sal e, dependendo do estágio da doença, a prática de atividades físicas regulares (ROSSIGNOL et al., 2019). Além disso, existe um grande avanço no desenvolvimento de pesquisas utilizando espécies vegetais ou outros produtos naturais. De fato, a ideia central é descobrir novas moléculas ou fitocomplexos úteis para o tratamento da IC.

2.4 Plantas medicinais

O termo plantas medicinal inclui vários tipos de espécies vegetais utilizadas na fitoterapia, sendo que centenas dessas plantas possuem atividades importantes para o tratamento de doenças (JAMSHIDI-KIA et al., 2018). Estima-se que cerca de 25% dos medicamentos atualmente comercializados são derivados de espécies vegetais (LIPEROTI et al., 2017). Historicamente, a fitoterapia sempre esteve envolvida em aliviar o sofrimento humano, e seu uso ainda cresce em todo o mundo. Esse crescimento, em parte, está relacionado à incapacidade da medicina moderna em enfrentar com sucesso a cronicidade de muitas doenças (SUROOWAN et al., 2015).

Devido a extraordinária diversidade de seus metabólitos as plantas oferecem uma fonte única e renovável para a descoberta de potenciais novos medicamentos, sendo que esses podem servir não apenas como novos fármacos, mas também como moléculas viáveis para otimização de fármacos já existentes (KURUPPU et al., 2019).

De fato, a variabilidade dos metabólitos secundários encontrado nos produtos naturais serviu como pilar histórico para o desenvolvimento de diversos fármacos utilizados no tratamento das DCV (SUROOWAN et al., 2015). Exemplo disso é o caso da digoxina e da digitoxina - derivadas de *Digitalis lanata* e *Digitalis purpúrea* - empregadas no tratamento da IC de baixo débito. Além disso, podemos citar a reserpina - alcaloide derivado da *Rauwolfia serpentina* – um importante anti-hipertensivo outrora comercializado, e a atropina - derivada da *Atropa belladonna* -, eficaz no tratamento de arritmias e bradicardia sinusal severa (LOBAY, 2015; KOHNEN-JOHANNSEN et al., 2019; SHAH et al., 2019).

Nesse contexto, ao levarmos em consideração a biodiversidade brasileira, observa-se

que a exploração dessa riqueza natural pode ser vista com um futuro favorável no tratamento de doenças que acometem o sistema cardiovascular.

2.5 *Plinia cauliflora* (Mart.) Kausel

Plinia cauliflora (Mart.) Kausel **(Figura 2)** é uma planta da família Myrtaceae, que possui mais de 120 gêneros. No Brasil, é conhecida popularmente como "jaboticabeira", "jaboticaba" ou "jabuticaba". Apresenta diferentes sinonímias incluindo *Eugenia cauliflora* DC., *Eugenia jaboticaba* (Vell.) Kiaersk., *Myrcia jaboticaba* (Vell.) Baill., *Myrciaria cauliflora* (Mart.) O. Berg, *Myrciaria jaboticaba* (Vell.) O. Berg, *Myrtus cauliflora* Mart., *Myrtus jaboticaba* (Vell.), e *Plinia jaboticaba* (Vell.) Kausel (LORENZI, 1998).

É uma árvore semidecídua, de 3 a 6 m, podendo chegar a 15 m de altura, com casca lisa de cor pardo-clara e manchada. Possui folhas glabras, com pontuações esparsas, de 3 a 7 cm de comprimento. As flores são aglomeradas sobre caule e ramos, sendo formadas na primavera e no verão. Os frutos globosos de polpa suculenta e doce são consumidos principalmente '*in natura*' sendo muito apreciados na forma de licor e geleia (LORENZI, 1998).

Figura 2: Exemplar e distribuição geográfica de *Plinia cauliflora* (Mart.) Kausel. (Fonte: Adaptado de https://www.gbif.org/species/5415571. Acesso em: 03 Jan. 2020.

Vários compostos fenólicos, incluindo flavonoides (sobretudo as antocianinas) presentes no fruto de *P. cauliflora* podem ser responsáveis por seus inúmeros efeitos biológicos **(Tabela 1)** (CHANG et al., 2019; GASPAROTTO JUNIOR et al., 2019). Sabe-se que, nos últimos anos, o uso de diferentes polifenóis tornou-se prática comum no tratamento de várias doenças, incluindo condições crônicas e neurodegenerativas, bem como em DCV (REIS et al., 2016). As antocianinas são um subgrupo de pigmentos solúveis em água responsáveis por dar coloração que varia do vermelho ao azul nas mais variadas plantas, flores, sementes, frutas e outros tecidos

vegetais (WALLACE et al., 2016).

Estudos têm demonstrado os efeitos benéficos desses polifenóis na DCV, inibindo o processo inflamatório, a disfunção endotelial e aumentando a produção de óxido nítrico (LI et al., 2017; VALENZA et al., 2018; KALT et al., 2019). Além disso, foi demonstrado que dietas ricas em polifenóis protegem contra doenças cardiovasculares associadas com o estresse oxidativo, como o IAM e a doença arterial coronariana (ISAAK et al., 2017). Entretanto, não há pesquisas que evidenciem a segurança farmacológica (SF) e o efeito cardioprotetor de *P. cauliflora*.

Parte da planta	**Uso medicinal**	**População**	**Referência**
Casca	Asma, diarreia, dor de garganta[a]; hemorragia pélvica, feridas uterinas, corrimento vaginal[b;]	Não informado[a]; População ribeirinha[b];	Cruz; Kaplan (2004[a]); Paiva et al. (2017[b]);
Folhas	Febre, diarreia[a]; diarreia, labirintite[b]; diarreia[c]; hemorragia pélvica, feridas uterinas[d]; diarreia broquite[e];	Curandeiros[a]; Comunidade rural[b,c]; População ribeirinha[d]; Curandeiros[e];	Maciel; Neto (2006[a]); Bieski et al., (2015[b]); Zeni; Bosio (2011[c]); Paiva et al., (2017[d]); Agra et al., (2008[e]);
Fruto	Alimento[a,b,c,d,e,f,g,]; diarreia[h];	Comunidade rural[a,f,g, h]; Comunidade urbana[b]; Não informado[c]; Comunidade costeira[d,e];	Pasa et al., (2008a); Amaral; Neto (2008[b]); Albuquerque et al., (2009[c]); Figueiredo et al., (2018[d]); Leal et al. (2018[e]); Bortolotto et al., (2015[f]); Do Nascimento et al., (2013[g]); Zeni; Bosio (2011[h]);
Casca do fruto	Diarreia[a];	Comunidade quilombola[a]	Silva et al., (2012[a])

Tabela 1: Uso medicinal de *P. cauliflora* e suas sinonímias (Fonte: Adaptado de Gasparotto Junior et al., 2019).

2.6 Segurança farmacológica

Estudos farmacológicos não-clínicos, como os que envolvem a SF, são elementos imprescindíveis ao longo do processo de descoberta e desenvolvimento de novas medicamentos (ANVISA, 2013). A SF tem como intuito principal a identificação dos possíveis efeitos farmacodinâmicos indesejáveis sobre as funções fisiológicas em diferentes sistemas

(VALENTIN et al., 2008). Nesse tipo de estudo, são avaliadas as funções da tríade composta pelo sistemas nervoso central, cardiovascular e respiratório (BASS et al., 2004).

A condução de estudos de SF se destaca pelo fato de que muitas drogas apresentam efeitos adversos tanto na função respiratória como na função cardíaca. Mudanças respiratórias, induzidas por drogas, podem ocorrer de maneira rápida e com alto risco de morte (VARGAS et al., 2013). Assim, as análises da função respiratória baseiam-se na frequência e na profundidade das trocas gasosas (MURPHY, 2002). Por outro lado, a avaliação da segurança sobre o sistema cardiovascular é estimada a partir de parâmetros como a PA, FC e a atividade elétrica cardíaca (GUTH et al., 2009). Já os efeitos da substância sobre o sistema nervoso central são avaliados apropriadamente por meio da presença de modificações comportamentais e motoras, por meio da bateria de observação funcional ou pelo teste de Irwin modificado (ANVISA, 2013).

3. OBJETIVOS

GERAL

Avaliar a segurança farmacológica e investigar os efeitos cardioprotetores do extrato etanólico obtido das cascas dos frutos maduros de *Plinia cauliflora* (Mart.) Kausel (EEPC) em coelhos Nova Zelândia.

ESPECÍFICOS

- Padronizar o melhor processo extrativo a partir das cascas dos frutos maduros de *P. cauliflora* e realizar caracterizações fitoquímicas;
- Avaliar os efeitos do EEPC nas doses de 200 mg/kg e 2000 mg/kg sobre os sistemas respiratório, cardiovascular e nervoso central de coelhos Nova Zelândia machos;
- Avaliar os efeitos do EEPC nas doses de 75 mg/kg e 150 mg/kg em coelhos fêmeas da linhagem Nova Zelândia portadores de IC;
- Avaliar as alterações eletrocardiográficas;
- Verificar as alterações de responsividade vascular renal;
- Mensurar o peso cardíaco absoluto e relativo;
- Verificar o perfil de marcadores bioquímicos séricos;
- Realizar análises anatopatológicas e morfométricas do músculo ventricular;
- Analisar os parâmetros do estresse oxidativo em amostras de soro e tecidos cardíaco e renal.

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5. APÊNDICES

5.1 **Artigo 1:** Pharmacological safety of *Plinia cauliflora* (Mart.) Kausel in rabbits

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Pharmacological safety of *Plinia cauliflora* (Mart.) Kausel in rabbits

Rhanany Alan Calloi Palozi[a], Lucas Pires Guarnier[a], Paulo Vitor Moreira Romão[a], Samara Requena Nocchi[b], Carlos Calixto dos Santos[c], Emerson Luiz Botelho Lourenço[c], Denise Brentan Silva[b], Francielly Mourão Gasparotto[a], Arquimedes Gasparotto Junior[a,*]

[a] *Laboratório de Eletrofisiologia e Farmacologia Cardiovascular (LEFaC), Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados, MS, Brazil*

[b] *Laboratório de Produtos Naturais e Espectrometria de Massas (LaPNEM), Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição (FACFAN), Universidade Federal do Mato Grosso do Sul, Campo Grande, MS, Brazil*

[c] *Laboratório de Pesquisa Pré-Clínica de Produtos Naturais, Universidade Paranaense, Umuarama, PR, Brazil*

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ABSTRACT

Fruit peels of *Plinia cauliflora* (Mart.) Kausel are widely used in Brazilian traditional medicine, but no studies have proved the safety of its pharmacological effects on the respiratory, cardiovascular, and central nervous systems. The present study assessed the safety pharmacology of *P. cauliflora* in New Zealand rabbits. First, an ethanol extract (EEPC) was selected for the pharmacological experiments and chemical characterization. Then, different groups of rabbits were orally treated with EEPC (200 and 2000 mg/kg) or vehicle. Acute behavioral and physiological alterations in the modified Irwin test, respiratory rate, arterial blood gas, and various cardiovascular parameters (i.e., heart rate, blood pressure, and electrocardiography) were evaluated. The main secondary metabolites that were identified in EEPC were ellagic acid, gallic acid, *O*-deoxyhexosyl quercetin, and the anthocyanin *O*-hexosyl cyanidin. No significant behavioral or physiological changes were observed in any of the groups. None of the doses of EEPC affected respiratory rate or arterial blood gas, with no changes on blood pressure or electrocardiographic parameters. The present study showed that EEPC did not cause any significant changes in respiratory, cardiovascular, or central nervous system function. These data provide scientific evidence of the effects of this species and important safety data for its clinical use.

1. Introduction

Safety pharmacology studies are essential for the development of new medicines [1]. This type of study aims to investigate the probable undesirable pharmacodynamic effects of new compounds on physiological functions, using doses to the therapeutic range and above [2]. One of the reasons that lead to pharmacological safety studies is due to serious adverse effects, especially on the central nervous system, respiratory rate, arterial blood gas (GAC), and cardiovascular parameters, including heart rate, blood pressure and cardiac electrical activity [3,4].

The Brazilian population, similar to populations worldwide, uses different vegetal species as important sources of food and medicine. Despite the widespread use of these agents, safety pharmacology studies are relatively restricted and mainly limited to compounds with broad industrial use. One example in Brazil is *Plinia cauliflora* (Mart.) Kausel (Myrtaceae). This species is endemic in South America. Its fruits are found in the most diverse Brazilian biomes, such as Cerrado, Caatinga, Atlantic Forest, Amazon Forest, and Pampa [5].

Popularly known as “jabuticaba,” the fruit is consumed fresh or used

Abbreviations: ABG, Arterial blood gas; ANOVA, One-way analysis of variance; ASE, Accelerated solvent extraction; BB, Buffer Base; BE, Base Excess; BE_{ecf}, Base excess in the extracellular fluid compartment; Ca^{++}, Calcium; $_cHCO_3$, Bicarbonate concentration; Cl, Chloride; CNS, Central nervous system; $_{ct}CO_2$ (B), Concentration of total carbon dioxide of whole blood; $_{ct}CO_2$ (P), Concentration of total carbon dioxide in plasma; $_{ct}O_2$, Concentration of total oxygen; DBP, Diastolic blood pressure; ECG, Electrocardiography; EEPC, Ethanol extract of *Plinia cauliflora*; GAE, Gallic acid equivalent; H^+, Hydrogen ion dissociated; Hct, Hematocrit; HHb, Deoxyhemoglobin; K^+, Potassium; LA, Left arm; LC-DAD-MS, Liquid chromatography coupled to a diode array detector and mass spectrometer; LL, Left leg; MAP, Mean arterial pressure; Na^+, Sodium; Na_2CO_3, Sodium carbonate; O_2Hb, Oxyhemoglobin; P50, Half of the maximum hemoglobin saturation; PCO_2, Partial pressure of carbon dioxide; pH, Potential of hydrogen; PO_2, Partial pressure of oxygen; RA, Right arm; RL, Right leg; S.E.M, Standard error of the mean; SBP, Systolic blood pressure; SO_2, Level of hemoglobin-saturation by oxygen; tHb, Hemoglobin; UFLC, Ultra fast liquid chromatograph

* Corresponding author.
E-mail address: arquimedesjunior@ufgd.edu.br (A. Gasparotto Junior).

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for the production of liqueur, vinegar, wine, juice, jam, and jelly [6]. Several phenolic compounds, including flavonoids and anthocyanins, are present in the fruit peel of *P. cauliflora* that exert numerous biological effects [7]. Several pharmacological studies have been conducted using different extracts that were obtained from fruit peels of this species, highlighting its antioxidant [8], hypotensive [9], anti-obesity [10], anti-inflammatory [11], hypolipidemic [12], and antibacterial [13] activity.

Despite the widespread use of *P. cauliflora* fruit peel in the production of different bioactive materials, no data are available in the literature on the safety pharmacology of this preparation. The present study sought to optimize extraction procedures for *P. cauliflora* fruit peel and perform a detailed phytochemical analysis. We also performed a detailed pharmacological safety study to evaluate respiratory, cardiovascular, and central nervous system effects in rabbits.

2. Material and methods

2.1. Phytochemical study

2.1.1. Plant material

Plinia cauliflora fruits were collected in Esperança Nova, Paraná, Brazil (-23.719864, -53.802104), in September 2017. A voucher specimen (no. 5983) was authenticated by Dr. Zefa Valdivina Pereira and deposited in the Herbarium of the Federal University of Grande Dourados (UFGD). The fruit peels were manually removed and dried by forced air circulation for 5 days. The dried peels were then pulverized in a knife mill and stored in plastic bags under refrigeration (2–8 °C) until use.

2.1.2. Extraction procedures by accelerated solvent extraction

The extract was obtained from peels by accelerated solvent extraction (ASE; Dionex) using the solvents acetone: water (1: 1, v/v), ethanol, ethanol: water (7: 3, v/v), and water. Nitrogen was used for ASE. The following parameters were applied and repeated three times: 125 °C temperature, 4 min static extraction time, 100% washing volume, 1500 psi pressure, and 60 s purge. The solvents were evaporated by a rotary evaporator (Büchi R-3, Flawil, Switzerland) under reduced pressure and lyophilized to yield the extracts. All of the extracts were analyzed by liquid chromatography-diode array detector-mass spectrometry (LC-DAD-MS), and the total phenol and tannin contents were determined.

2.1.3. Total phenolic content

The phenolic content determination was based on the methodology of Herald et al. (2012) [14] with minor modifications. A 96-well microplate was used. To each well were added 75 µl of methanol and 75 µl of sample or standard (gallic acid), which were used for serial dilutions. Folin-Ciocalteu reagent (1:1 v/v, deionized water) was then added to the wells. After 6 min, 75 g/l (100 µl) Na_2CO_3 was added and mixed again. After 90 min, the samples were measured at 765 nm using a spectrophotometric microplate reader. The analyses were performed in triplicate. The results are expressed as milligrams (mg) of gallic acid equivalent (GAE) per gram (g) of extract.

2.1.4. Total tannin content

The extracts were solubilized at a concentration of 4 mg/ml using methanol and water (1:1, v/v), and skin powder (20 mg) was added and stirred for 60 min. After centrifugation, the supernatants were used for total phenol content determination as described previously. Total tannin content was calculated as the difference between the concentration of total phenols and non-tannin phenols. The results are expressed as milligrams of GAE per gram of extract.

2.1.5. Antioxidant activity determined by DPPH assay

The DPPH assay was performed according to Fukumoto and Mazza (2000) [15] with minor modifications. A 96-well microplate was used. A 150-µM solution of DPPH was prepared in methanol: water (8: 2 v/v). For each well was added 200 µl of DPPH solution, with the exception of blank wells, to which only methanol: water (8: 2 v/v) was added. Samples were analyzed in triplicate for each concentration (0–500 µg/ml). In control, only DPPH solution and methanol: water (8:2 v/v) were added. Quercetin was applied as the standard. The plate was covered and left in the dark at room temperature. After 6 h, absorbance was read at 520 nm in a spectrophotometric microplate reader. Absorbance decay of the samples (Aam) that is correlated with absorbance decay of the control (Ac) results in a percentage of free radical sequestration (% FRS):

$$\% \text{ FRS} = (\text{Ac} - \text{As}) / \text{Ac} \times 100$$

The data were used to calculate the IC_{50} (the concentration that is able to sequester 50% of free radicals).

2.1.6. Identification of constituents in the extracts determined by LC-DAD-MS

A UFLC Prominence Shimadzu LC device coupled to a DAD and MicrOTOF-Q III mass spectrometer (Bruker Daltonics, Billerica, MA, USA) was used for the analyses. A Kinetex C18 column (2.6 µm, 150 mm × 2.1 mm, Phenomenex) was applied, with a 1 µl injection volume, 0.3 ml/min flow rate, and 50 °C oven temperature. For the mobile phase, water (A) and acetonitrile (B) were used, to which 0.1% formic acid (v/v) was added. The gradient elution profile was the following: 3% B for 0–2 min, 3–25% B for 2–25 min, 25–80% B for 25–40 min, and 80% B for 40–43 min, followed by subsequent reconditioning conditions (5 min). The analyses were performed in negative and positive ion modes. Nitrogen was applied as the nebulizer gas at 4 bar and drying gas at 9 L/min. The capillary voltage was 3.5 kV. All of the extracts were prepared at 1 mg/ml, filtered (Millex0.22 µm, PTFE, Millipore), and injected in the chromatographic system.

The compounds were identified based on ultraviolet spectra, accurate mass, and fragmentation profile and compared with data in the literature. The molecular formulas were determined by accurate mass, considering errors up to 8 ppm and mSigma < 25.

2.2. Safety pharmacology study

2.2.1. Animals

Male New Zealand rabbits (Twenty-week-old) were obtained from Universidade Federal do Paraná (UFPR, Brazil) and housed in the vivarium of UFGD under controlled temperature (20 °C ± 2 °C) and humidity (50% ± 10%) and a 12 h/12 h light/dark cycle with ad libitum access to food and water. The Institutional Ethics Committee of the Universidade Federal da Grande Dourados previously approved all procedures employed in this study (UFGD, Brazil; protocol no. 11/2018; approved March 16, 2018).

2.2.2. Effects on the central nervous system

The effects on the central nervous system were performed according to the modified Irwin test [16]. After a 6-h fasting, two doses of EEPC (200 and 2000 mg/kg) [17] were administered in different groups of male rabbits (n = 6/group) by oral gavage. Filtered water was administered in the control group (1 ml/kg; n = 6). Food was only given to the rabbits 1 h after treatment. The effects of the treatments were evaluated 0-15 min, 15, 30, 60, 120, and 180 min, and 24 h after the administrations. To observe possible behavioral and physiological changes, the following parameters were recorded: piloerection, stereotypes (i.e., chewing, sniffing, and head movements), scratching, catalepsy, locomotor activity, reactivity to touch, akinesia, head-twitches, tremors, jumping, aggression, gait (rolling, tip-toeing), fear-related behavior, motor coordination, convulsions, grasping, traction, writhing, analgesia, exophthalmia, mydriasis, ptosis, myosis, salivation,

lacrimation, diarrhea, defecation, respiration, hyperthermia, and hypothermia.

2.2.3. Cardiovascular and respiratory evaluation

The effects on the cardiovascular and respiratory systems were evaluated according to the adapted protocol of Grahan and Li (1973) [18]. After 6-h fasting, two doses (200 and 2000 mg/kg) of EEPC (17)] were administered in different groups of rabbits (n = 6/group) by oral gavage. Vehicle (filtered water) was administered in the control group (1 ml/kg).

2.2.3.1. Respiratory rate and ABG analysis. One hour after treatment, all of the rabbits remained conscious in the ventral decubitus position. Respiratory rate was determined using a Kofranyi-Michaelis respirometer [19]. For ABG analysis, arterial blood samples were obtained from the central artery of the ear and immediately processed. All of the parameters below were determined in a Cobas b 221 blood gas system (Roche Diagnostics, Rotkreuz, Switzerland): pH, PCO_2 (mmHg), PO_2 (mmHg), SO_2 (%), Hct (%), tHb (g/dl), Na^+ (mmol/l), K^+ (mmol/L), Ca^{2+} (mmol/L), Cl^- (mmol/L), glucose (mg/dl), lactate (mmol/L), O_2Hb (%), HHb (%), P50 (mmHg), H^+ (nmol/L), BE (nmol/L), BE_{ecf} (nmol/L), BB (mmol/L), $cHCO_3$ (mmol/L), $ctCO_2$ (B) (mmol/L), $ctCO_2$ (P) (mmol/L), and ctO_2 (vol%).

2.2.3.2. Electrocardiography. After evaluating the respiratory system, all of the rabbits were intramuscularly anesthetized with 10-mg/kg diazepam plus 115-mg/kg ketamine and kept in the dorsal decubitus position. Four alligator electrodes (RL, RA, LL, and LA) were positioned in the folds of both knees and elbows. A small amount of conductive gel was applied to each electrode for better electrical conduction. In addition, 6 (V1-V6) precordial electrodes were also connected. The V1 was placed in the fourth intercostal space, on the right margin of the sternum. The V2 was placed in the fourth intercostal space, on the left margin of the sternum. The V3 is halfway between the electrodes V2 and V4. The V4 in the fifth left intercostal space, in the hemiclavicular line. The V5 is at the same level as the electrode V4, in the anterior axillary line. And the V6 was placed on the same level as the electrodes V4 and V5, on the mid-axillary line. After 5 min for acclimation, the electrocardiographic waves were recorded for 5 min in an ECG recorder (WinCardio, Micromed, Brasília, Brazil).

2.2.3.3. Effects on blood pressure. After electrocardiography, all of the rabbits subcutaneously received a bolus injection of heparin (50 IU). A tracheotomy was then performed to allow the animals to breathe spontaneously. The left carotid artery was then isolated, cannulated, and coupled to a pressure transducer connected to a PowerLab recording system. Chart 4.1 (ADI Instruments, Castle Hill, Australia) was used to register diastolic blood pressure (DBP), systolic blood pressure (SBP), and mean arterial pressure (MAP). Changes in SBP, DBP, and MAP were recorded for 20 min.

2.3. Statistical analysis

The mean ± standard error of the mean (SEM) is shown. Differences between groups were assessed using analysis of variance (ANOVA), followed by Dunnett's post hoc test. Values of $p < 0.05$ were considered statistically significant. GraphPad Prism Mac 6.0 was used to draw the graphs and for statistical analysis.

3. Results

3.1. Optimization of extraction procedures

The extracts were obtained by ASE using the solvents ethanol, ethanol:water (7:3), acetone:water (1:1), and water. All of the extracts were analyzed by LC-DAD-MS (Fig. 1), and total phenol content, tannin content, and antioxidant activity were determined (Table 1).

The extracts that were obtained with acetone:water (1:1) and ethanol:water (7:3) had the highest total phenol content and tannin content and high antioxidant activity, with an IC_{50} of 13.08 ± 2.03 µg/ml and 11.54 ± 0.20 µg/ml, respectively. The extracts that were obtained with acetone:water (1:1) and ethanol:water (7:3) had total phenol content of 313.86 ± 1.73 mg GAE/g and 299.60 ± 4.26 mg GAE/g, respectively, and tannin content of 169.64 ± 6.74 mg GAE/g and 179.46 ± 1.76 mg GAE/g, respectively. The chromatograms of the extracts revealed chemical similarities (Fig. 1), but ion peak intensities presented some differences. More chromatographic peaks were observed with the ethanol:water (7:3) extract. Overall, the extracts that were obtained with acetone:water (1:1) and ethanol:water (7:3) presented the best results. The ethanol:water extract was selected for the safety pharmacology study because this solvent composition is widely used because of its low toxicity.

3.2. Identification of extract constituents determined by LC-DAD-MS

The ethanol:water (7:3) extract (EEPC) was analyzed by LC-DAD-MS, and 12 compounds were identified (Table 2, Fig. 2). Compounds **1** and **2** showed intense ions at m/z 341.1083 and 191.0181 $[M\text{-}H]^-$, which were putatively identified as di-hexoside and citric acid, respectively. Peak **3** revealed a band at a wavelength of 270 nm in the ultraviolet spectrum and also an ion at m/z 169.0121 $[M\text{-}H]^-$, which was confirmed as gallic acid by injection of the authentic standard. This compound has been previously reported in *P. cauliflora* (13).

Compounds **7**-**10** showed bands in the ultraviolet spectra at wavelengths of 260 and 360 nm, suggesting a chromophore group relative to ellagic acid. Compound **9** presented the molecular formula $C_{14}H_6O_8$ (from ions m/z 301.9979 and 303.0157) and fragment ions at m/z 283, 257, and 229, which resulted from losses of water, CO_2, and CO molecules. This fragmentation pathway is similar to the ellagic acid profile [20,21], which was confirmed by injection of the authentic standard. Compounds **7** (m/z 465.0677, $C_{20}H_{17}O_{13}^+$), **8** (m/z 435.0575, $C_{19}H_{15}O_{12}^+$), and **10** (m/z 449.0729, $C_{20}H_{17}O_{12}^+$) presented fragment ions at m/z 303 from losses of hexosyl (162 *u*), pentosyl (132 *u*), and deoxyhexosyl (146 *u*) groups. Compounds **7**, **8**, and **10** were identified as *O*-hexosyl ellagic acid, *O*-pentosyl ellagic acid, and *O*-deoxyhexosyl ellagic acid, which have been previously reported in *Plinia* species (*P. trunciflora*, *P. caulifora*, *P. jaboticaba*, and *P. phitrantha*) [20].

Compounds **6** and **11** showed characteristic ultraviolet spectra of anthocyanin (λ_{max} = 279 and 512 nm) and flavonols (λ_{max} = 265 and 358 nm; Markham, 1982) [22]. Ions at m/z 449.1098 $[M]^+$ and 449.1097 $[M+H]^+$ confirmed the molecular formulas $C_{21}H_{21}O_{11}^+$ and $C_{21}H_{20}O_{11}$, respectively. The loss of 162 *u* indicated their hexosyl substituents. Compounds **6** and **11** were identified as *O*-hexosyl cyanidin and *O*-deoxyhexosyl quercetin, respectively, which was similar to data reported by Calloni et al. (2015) [23] and Neves et al. (2018) (20) from *Plinia* species.

3.3. Effects on the central nervous system

The toxic effects of EEPC on behavioral and physiological status in male rabbits are shown in Table 3. During the 24 h observation period, none of the experimental animals were inactive or refused to consume food or water. No significant changes in behavior or physiological status were observed until the end of 24 h (i.e., convulsions, tremors, locomotor activity, jumping, fear-related behavior, reactivity to touch, aggression, head-twitches, stereotypies [i.e., head movements, chewing, sniffing], scratching, catalepsy, akinesia, gait [rolling, tip-toeing], motor coordination, traction, grasping, writhing, analgesia, ptosis, exophthalmia, myosis, mydriasis, piloerection, defecation, diarrhea, salivation, lacrimation, respiration, hypothermia, and hyperthermia).

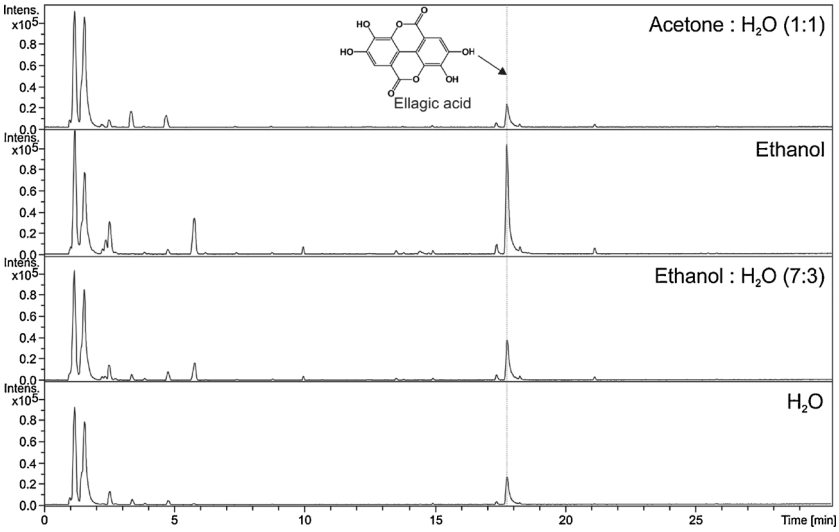

Fig. 1. Base peak chromatograms (negative ion mode) from the extracts of peels obtained by ASE with the extractor solvents acetone:water (1:1 v/v), ethanol and ethanol:water (7:3 v/v) and water.

Table 1
. Yield, phenolic content, tannin content and IC_{50} value for free radical scavenging activity by DPPH method from the extracts.

Extract	Yield (%)	Phenolic content (mg GAE g^{-1})	Tannin content (mg GAE g^{-1})	DPPH IC_{50} (μg/mL)
Acetone:water (1:1)	27.9	313.86 ± 1.73	169.64 ± 6.74	13.08 ± 2.03
Ethanol	24.5	197.39 ± 5.71	69.05 ± 5.23	16.25 ± 1.18
Ethanol:water (7:3)	22.6	299.60 ± 4.26	179.46 ± 1.76	11.54 ± 0.20
Water	16.8	181.42 ± 3.67	136.13 ± 6.58	23.75 ± 0.72
Quercetin	–	–	–	1.73 ± 0.10

GAE: gallic acid equivalent (GAE) g^{-1} (per gram) of extract.

Table 2
Identification of the constituents from ethanol and water (7:3) extract (EEPC) of *P. cauliflora* by LC-DAD-MS.

Peak	RT (min)	Compound	MF	UV (nm)	Negative mode (*m/z*)		Positive mode (*m/z*)	
					MS $[M-H]^-$	MS/MS	MS $[M+H]^+$	MS/MS
1	1.2	di-hexoside	$C_{12}H_{22}O_{11}$	–	341.1083	191	–	–
2	1.6	Citric acid	$C_6H_8O_7$	–	191.0181	–	–	–
3	2.5	Gallic acid*	$C_7H_6O_5$	270	169.0121	–	–	–
4	3.0	NI	$C_6H_{10}N_6O_4$	–	–	–	231.0842	
5	5.8	NI	$C_8H_{12}O_7$	–	219.0492	–	221.0659	–
6	12.5	*O*-hexosyl cyanidin	$C_{21}H_{21}O_{11}^+$	279, 512	447.0933	284, 255, 162, 147	449.1098	287
7	14.9	*O*-hexosyl ellagic acid	$C_{20}H_{16}O_{13}$	255, 360	463.0513	–	465.0677	303
8	17.3	*O*-pentosyl ellagic acid	$C_{19}H_{14}O_{12}$	265, 360	433.0411	301	435.0575	303
9	17.7	Ellagic acid*	$C_{14}H_6O_8$	255, 362	301.9979	283, 257, 229, 185	303.0157	275, 257, 229, 201
10	18.3	*O*-deoxyhexosyl ellagic acid	$C_{20}H_{16}O_{12}$	265, 365	447.0577	301	449.0729	303
11	21.1	*O*-deoxyhexosyl quercetin	$C_{21}H_{20}O_{11}$	265, 358	447.0929	300, 271, 255, 243, 178,163	449.1097	303
12	35.9	NI	$C_{13}H_{18}O_4$	279	237.1134	221, 206, 166	–	–

RT: retention time; MF: molecular formula; NI: non-identified; *confirmed by the authentic standard.

3.4. Respiratory rate and ABG analysis

The mean respiratory rate in rabbits that were treated with vehicle alone was 57 ± 6.01. No increase or decrease in respiratory rate was observed after acute EEPC administration (200 mg/kg EEPC: 56 ± 7.58; 2000 mg/kg EEPC: 55 ± 6.59). The ABG analysis indicated that none of the doses of EEPC altered pH, PCO_2 (mmHg), PO_2 (mmHg), SO_2 (%), Hct (%), tHb (g/dl), Na^+ (mmol/L), K^+ (mmol/L), Ca^{2+} (mmol/L), Cl^- (mmol/L), glucose (mg/dl), lactate (mmol/L), O_2Hb (%), HHb (%), P50 (mmHg), H^+ (nmol/L), BE (nmol/L), BE_{ecf} (nmol/L), BB (mmol/L), $cHCO_3$ (mmol/L), $ctCO_2$ (B) (mmol/L), $ctCO_2$ (P) (mmol/L), or ctO_2 (vol%) compared with control animals (Table 4).

3.5. Electrocardiography

Fig. 3A-G shows representative electrocardiograms and quantitative data for rabbits that were treated with 200 and 2000 mg/kg EEPC or vehicle. We did not observe any significant changes in electrocardiographic characteristics (PR, QRS, or QT segment) between experimental groups, with no alterations of the amplitude of P-, R-, or T-waves.

3.6. Effects on blood pressure

Basal SBP, DBP, and MAP that were recorded after the 15-min stabilization period were 102 ± 6.6 mmHg, 62 ± 3.5 mmHg, and 71 ± 4.1 mmHg, respectively, in control animals. Oral administration

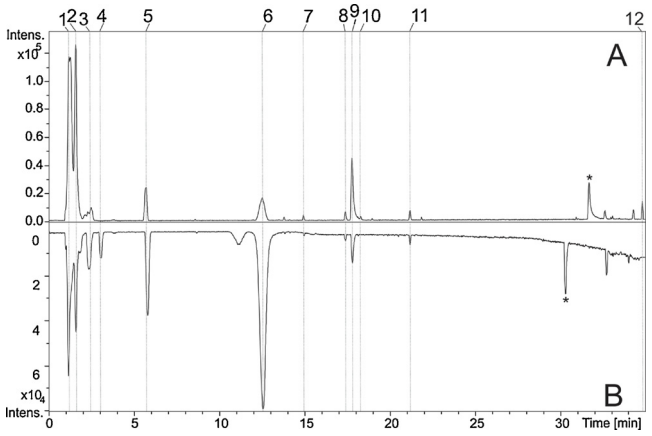

Fig. 2. Base peak chromatograms obtained by negative (**A**) and positive ion modes (**B**) from ethanol:water (7:3) extract (EEPC). (*chromatographic peaks are not of the analyzed sample).

Table 3
Effects of EEPC acute treatment on behaviors and clinical signals observed in Irwin modified test.

Category	Symptoms	Control	EEPC (200 mg/kg)	EEPC (2000 mg/kg)
Excitation	Convulsion	(-)	(-)	(-)
	Tremor	(-)	(-)	(-)
	Increased activity	(-)	(-)	(-)
	Jumping	(-)	(-)	(-)
	Increase fear	(-)	(-)	(-)
	Increased reactivity to touch	(-)	(-)	(-)
	Aggression	(-)	(-)	(-)
Stereotypy	Head-twitches	(-)	(-)	(-)
	Stereotypies (head movements)	(-)	(-)	(-)
	Stereotypies (chewing)	(-)	(-)	(-)
	Stereotypies (sniffing)	(-)	(-)	(-)
	Scratching	(-)	(-)	(-)
Motor	Catalepsy	(-)	(-)	(-)
	Akinesia	(-)	(-)	(-)
	Abnormal gait (rolling)	(-)	(-)	(-)
	Abnormal gait (tip-toe)	(-)	(-)	(-)
	Motor incoordination	(-)	(-)	(-)
	Loss of traction	(-)	(-)	(-)
	Grasping	(-)	(-)	(-)
Sedation	Decreased activity	(-)	(-)	(-)
	Decreased fear	(-)	(-)	(-)
	Decreased reactivity to touch	(-)	(-)	(-)
Pain	Writhing	(-)	(-)	(-)
	Analgesia	(-)	(-)	(-)
Autonomic	Ptosis	(-)	(-)	(-)
	Exophthalmia	(-)	(-)	(-)
	Myosis	(-)	(-)	(-)
	Midriasis	(-)	(-)	(-)
	Piloerection	(-)	(-)	(-)
	Defecation	(-)	(-)	(-)
	Diarrhea	(-)	(-)	(-)
	Salivation	(-)	(-)	(-)
	Lacrimation	(-)	(-)	(-)
Others	Increased respiration	(-)	(-)	(-)
	Decreased respiration	(-)	(-)	(-)
	Hypothermia	(-)	(-)	(-)
	Hyperthermia	(-)	(-)	(-)

The evaluation time was 0–15 min, 15, 30, 60, 120, 180 min and 24 h after the treatments acute administration. (-): Absence of the symptom.

of EEPC (200 or 2000 mg/kg) did not significantly change SBP, DBP, or MAP compared with the control group (Fig. 4A-C). Heart rate was not significantly different between experimental groups. The mean values

Table 4
Effects of EEPC acute treatment on respiratory rate, blood gases, electrolytes, and metabolites parameters.

	Control	EEPC (200 mg/kg)	EEPC (2000 mg/kg)
Respiratory rate	57 ± 6.01	56 ± 7.58	55 ± 6.59
Blood gases			
pH	7.25 ± 0.02	7.28 ± 0.04	7.25 ± 0.03
PCO_2 (mmHg)	55.90 ± 1.70	56.85 ± 2.42	56.42 ± 2.21
PO_2 (mmHg)	80.53 ± 3.13	71.83 ± 4.76	75.44 ± 4.51
SO_2 (%)	82.65 ± 6.31	88.78 ± 1.75	86.71 ± 1.88
Hct (%)	37.98 ± 2.71	38.25 ± 2.36	37.44 ± 2.51
tHb (g/dl)	13.85 ± 0.88	13.45 ± 1.13	13.55 ± 1.11
Electrolytes			
Na^+ (mmol/l)	143.10 ± 0.82	144.40 ± 0.85	142.23 ± 0.79
K^+ (mmol/l)	4.31 ± 0.12	4.35 ± 0.22	4.32 ± 0.18
Ca^{++} (mmol/l)	1.07 ± 0.02	1.04 ± 0.01	1.06 ± 0.02
Cl^- (mmol/l)	103.30 ± 0.68	104.50 ± 0.53	103.45 ± 0.61
Metabolites			
Glucose (mg/dl)	295.30 ± 18.93	303.80 ± 20.88	299.91 ± 21.31
Lactate (mmol/l)	1.05 ± 0.11	1.22 ± 0.22	1.12 ± 0.21
C)-oximetry			
O_2Hb (%)	88.75 ± 1.82	82.67 ± 3.59	85.13 ± 2.88
HHb (%)	14.15 ± 1.22	11.50 ± 1.59	13.20 ± 1.33
Calculated values			
P50 (mmHg)	38.03 ± 2.02	40.35 ± 0.99	39.22 ± 1.18
H^+ (nmol/l)	53.18 ± 5.71	52.58 ± 3.72	51.47 ± 2.99
BE (nmol/l)	−3.70 ± 0.47	−3.47 ± 0.28	−3.61 ± 0.33
BE_{ecf} (nmol/l)	−3.07 ± 0.77	−3.10 ± 0.14	−3.13 ± 0.41
BB (mmol/l)	43.80 ± 0.54	43.90 ± 0.63	43.65 ± 0.59
$cHCO_3$ (mmol/l)	93.68 ± 0.61	90.50 ± 1.78	92.45 ± 0.99
$_{ct}CO_2$ (B) (mmol/l)	23.63 ± 0.39	24.98 ± 0.92	23.44 ± 0.57
$_{ct}CO_2$ (P) (mmol/l)	21.55 ± 0.73	22.95 ± 0.76	21.35 ± 0.71
ctO_2 (vol%)	15.83 ± 1.04	15.38 ± 0.59	15.54 ± 0.85

Statistical analyses were performed using one-way ANOVA followed by Dunnett post hoc test. PCO_2: partial pressure of carbon dioxide, PO_2: partial pressure of oxygen, SO_2: level of hemoglobin-saturation by oxygen, Hct: hematocrit, tHb: hemoglobin, Na^+: sodium, K^+: potassium, Ca^{++}: calcium, Cl^-: chloride. O_2Hb: Ox hemoglobin, HHb: Deoxyhemoglobin, P50: half of the maximum hemoglobin saturation, H^+: hydrogen ion dissociated, BE: base excess, BE_{ecf}: base excess in the extracellular fluid compartment, BB: buffer base, $cHCO_3$: bicarbonate concentration, $_{ct}CO_2$ (B): concentration of total carbon dioxide of whole blood, $_{ct}CO_2$ (P): concentration of total carbon dioxide in plasma, ctO_2: concentration of total oxygen.

in the negative control and EEPC (200 and 2000 mg/kg) groups were 272 ± 11 bpm, 295 ± 23 bpm, and 284 ± 17 bpm, respectively (Fig. 4D).

4. Discussion

Natural products have been used as sources of food and as raw material for the production of medicines throughout history. Medicinal or fructiferous plants have been and remain to be used worldwide because of their economic and curative potential [24]. Although numerous pharmacological studies have investigated a wide range of new potential drugs, toxicological studies are not always carefully performed. Several data have reported potential side effects and systemic toxicity of several natural products [25]. In this sense, studies that evaluate pharmacological safety are important to ensure safe use and therapeutic rationalization. Thus, we obtained an ethanol extract from *Plinia cauliflora* fruit peels and mapped its main active metabolites. We found that the EEPC did not have significant deleterious effects on biological systems that are most affected by active molecules (i.e., cardiovascular, respiratory, and central nervous systems).

Different physiological systems of the body are subject to several deleterious effects of natural products. The respiratory, cardiovascular, and central nervous systems are key systems where such effects can occur, possibly with greater severity. Because of the high vascularization of these organs, biological agents can readily access them and reach target tissues rapidly and at high concentrations. Moreover, the

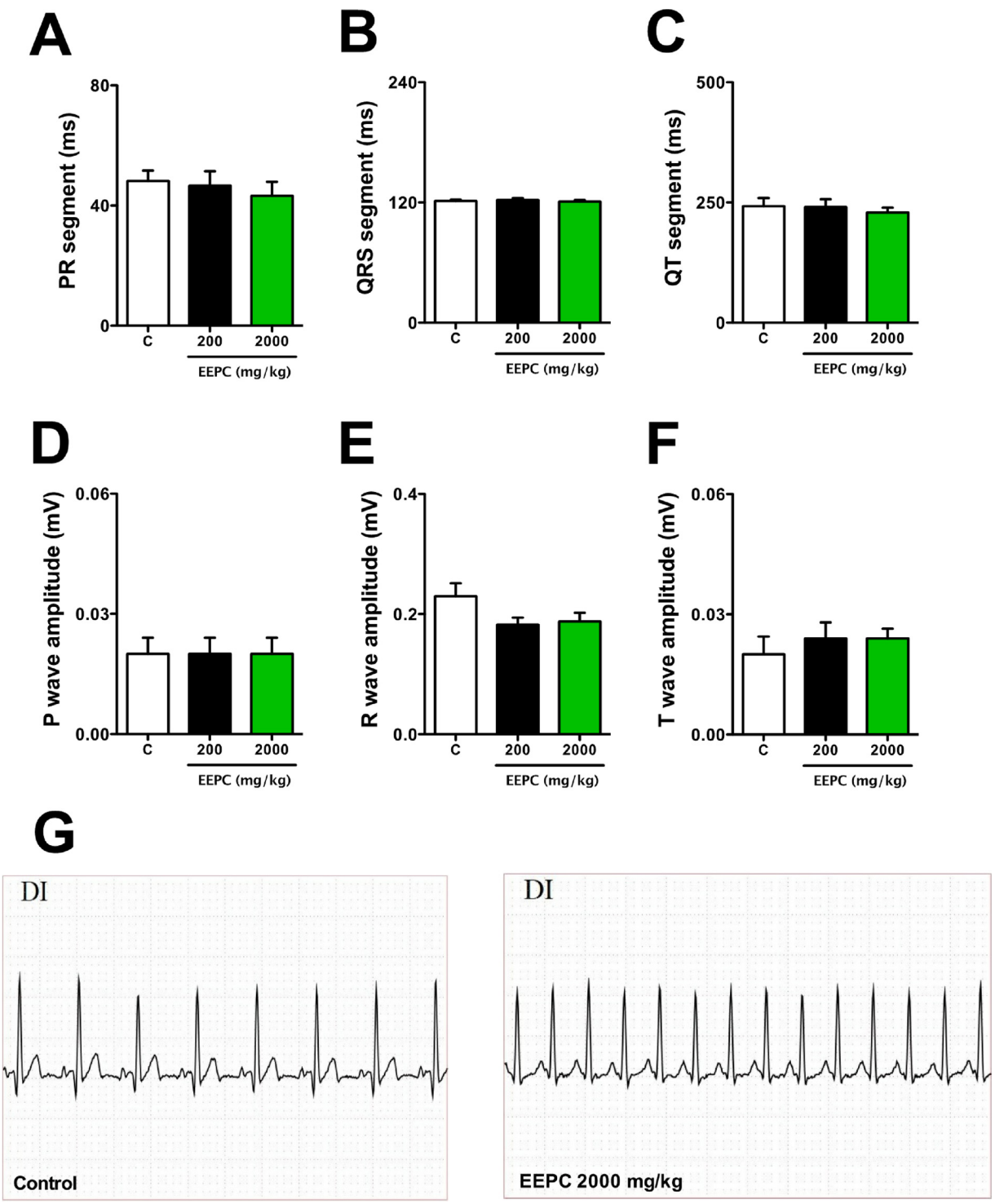

Fig. 3. Electrocardiographic quantitative data of rabbits treated with EEPC or vehicle in the PR (A), QRS (B), and QT-segments (C) and P (D), R (E) and T-waves (F) amplitude. Representative electrocardiographic records carried out in limb lead I also are shown (G). Statistical analyses were performed using one-way ANOVA followed by Dunnett *post hoc* test. The results are expressed as mean ± standard error of the mean (S.E.M.) and *p*-value of less than 0.05 was considered statistically significant. C: control (vehicle); EEPC: ethanol extract from *Plinia cauliflora*.

lungs, heart, and brain regulate highly sensitive bodily functions. Any changes that occur acutely in these organs can have significant consequences on body homeostasis [26].

When conducting preclinical safety pharmacology studies, an important factor to be considered is the animal model that is employed. Although rodents are a key pharmacological tool, they are not always a viable model for such pharmacological safety studies. Rats can be considered an acceptable option for studies of substances with potential effects on the central nervous system [27], including behavioral studies [28]. However, rats may be considered inadequate for evaluating effects on the cardiovascular system because the electrocardiographic tracing and arrhythmogenic potential of some drugs can have different profiles between rodents and humans [29,30]. The use of lagomorphs has gained prominence in this field. In addition to relatively easy maintenance and rapid reproduction, rabbits also possess physiological functions that are similar to humans.

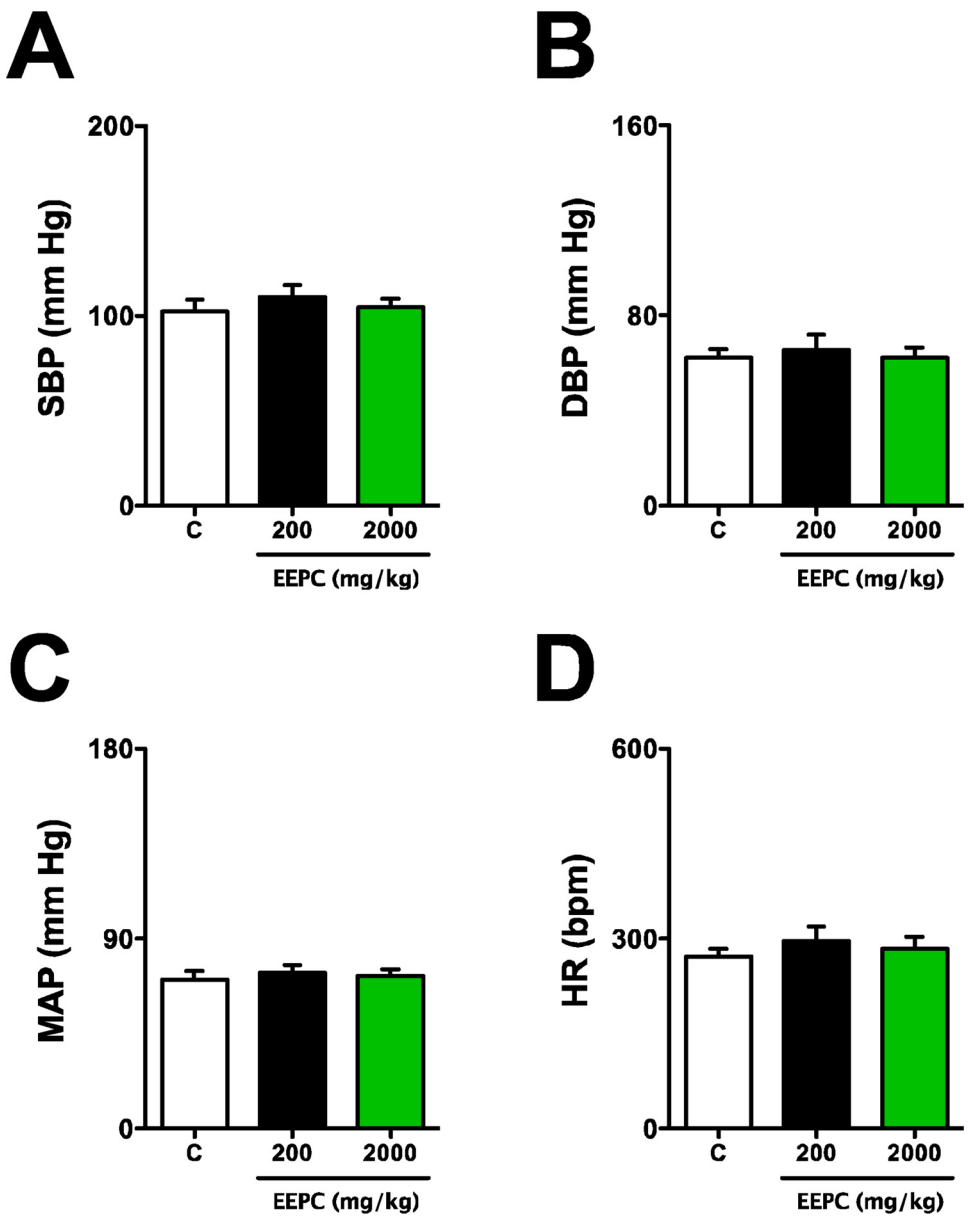

Fig. 4. Acute oral administration of EEPC obtained from *P. cauliflora* does not affect SBP, DBP, MAP, and HR in New Zealand rabbits. Statistical analyses were performed using one-way ANOVA followed by Dunnett *post hoc* test. The results are expressed as mean ± standard error of the mean (S.E.M.) and *p*-value of less than 0.05 was considered statistically significant. C: control (vehicle); DBP: diastolic blood pressure; EEPC: ethanol extract from *Plinia cauliflora*; HR: heart rate; MAP: mean arterial pressure; SBP: systolic blood pressure.

Several herbal medicines have significant action on the central nervous system. Many of them have also become important sources of modern drugs, including atropine (*Atropa belladonna* L.), morphine (*Papaver somniferum* L.), caffeine (*Coffea arabica* L.), and ephedrine (*Ephedra sinica* Stapf) [31–35]. Data also show that some species are still used as an abuse drug or due their recreational potential (e.g., *Erythroxylum coca* Lam. and *Cannabis sativa* L. [36–38]. Moreover, poisoning by natural products is not uncommon because of the sensitivity of the central nervous system to these agents [39,40]. Thus, the Irwin test is employed to evaluate the effects of a new drug on physiological and behavioral functions [41]; and may alert us to potential safety concerns, including seizure potential, sedation, and motor changes [42].

Another important fact to consider is the respiratory depressant potential of some natural products. In the United States alone, a significant increase in cases of opioid-induced fatal respiratory depression has been observed in recent decades [43,44]. In addition to more severe cases, deleterious effects on the respiratory system may present more subtly and become severe in the long term. Cases of respiratory acidosis or low oxygen saturation in red blood cells may be considered important indicators of systemic toxicity [45,46].

Cardiovascular effects are one of the least explored areas during safety evaluations of natural products. Although effects on blood pressure have been systematically evaluated, electrocardiographic profiles that are observed after treatment with different natural products have been quite unusual [47–49]. This can be concerning when considering the fact that some natural molecules are classic blockers of sodium channels (e.g., quinidine) in cardiac muscle [50,51]. Some studies have shown the ability of some drugs to affect the duration of the cardiac action potential. In fact, several drugs induce prolongation of the Q-T interval and may precipitate ventricular arrhythmias [52]. Any substance that is intended for long-term use, such as polyphenol-rich antioxidant preparations, should be evaluated for cardiovascular safety.

In recent years, consumers have sought treatments that are able to slow aging or prevent cardiovascular diseases. *P. cauliflora* has been used in Brazil for this purpose [53]. Their fruit peels are rich in polyphenols, such as anthocyanin, flavonoids, and ellagic acid derivatives; and these molecules are classically known as antioxidants [6–8,54,55]. Although we found that the EEPC was relatively safe with regard to its effects on the respiratory, cardiovascular, and central nervous systems, we do not exclude the possibility that other species with a similar phytochemical profile may present deleterious effects. In fact, several isolated secondary metabolites that are present in the EEPC have important effects on the central nervous system and different ion channels [56–60]. Thus, different extraction processes result in unique phytochemical profiles, and safety pharmacology studies need to be performed with each preparation of interest to guarantee their safety.

5. Conclusion

The present study found that the EEPC that was obtained from *Plinia cauliflora* fruit peels did not cause any significant changes in respiratory, cardiovascular, or central nervous system function. These findings provide important scientific knowledge about the species and safety data for its clinical use.

Author's contributions

All authors participated in the design, interpretation of the studies, analysis of the data and review of the manuscript; RACP, LPG, and PVMR conducted the experiments; DBS and SRN were involved with the preparation and chemical analysis of extract; ELBL, RACP and AGJ was responsible for data discussion, manuscript correction and AGJ was the senior researcher responsible for this work. All authors read and approved the final manuscript.

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5.2 **Artigo 2:** Cardioprotective effects of *Plinia cauliflora* (Mart.) Kausel in a rabbit model of doxorubicin-induced heart failure

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Cardioprotective effects of *Plinia cauliflora* (Mart.) Kausel in a rabbit model of doxorubicin-induced heart failure

Paulo Vitor Moreira Romão[a], Rhanany Alan Calloi Palozi[a], Lucas Pires Guarnier[a], Aniely Oliveira Silva[a], Bethânia Rosa Lorençone[a], Samara Requena Nocchi[b], Cátia Cristina de Freitas Sari Moura[c], Emerson Luiz Botelho Lourenço[c], Denise Brentan Silva[b], Arquimedes Gasparotto Junior[a,*]

[a] *Laboratório de Eletrofisiologia e Farmacologia Cardiovascular (LEFaC), Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados, MS, Brazil*

[b] *Laboratório de Produtos Naturais e Espectrometria de Massas (LaPNEM), Faculdade de Ciências Farmacêuticas, Alimentos e Nutrição (FACFAN), Universidade Federal do Mato Grosso do Sul, Campo Grande, MS, Brazil*

[c] *Laboratório de Pesquisa Pré-Clínica de Produtos Naturais, Universidade Paranaense, Umuarama, PR, Brazil*

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ABSTRACT

Ethnopharmacological relevance: In Brazil, the fruit of a native species that is popularly known as "jabuticaba" (*Plinia cauliflora* [Mart.] Kausel) is widely consumed fresh or used for the production of liqueur, juice, and jelly. In Brazilian folk medicine, this species is used to treat asthma, throat inflammation, and gastrointestinal and cardiovascular disturbances. However, no previous studies have reported its cardioprotective effects.

Aim: To evaluate the possible cardioprotective effects of a hydroethanolic extract of *Plinia cauliflora* (EEPC) in female rabbits in a model of doxorubicin-induced heart failure.

Material and methods: EEPC was obtained and fractionated by solid phase extraction, and its constituents were determined by liquid chromatography coupled to diode array detector and mass spectrometry (LC-DAD-MS). Thirty female New Zealand rabbits received doxorubicin administration for 6 weeks to induce heart failure. EEPC was orally administered at doses of 75 and 150 mg/kg daily for 42 days. Enalapril (5 mg/kg) was used as a reference cardioprotective drug. At the end of the experimental period, blood pressure and heart rate were recorded. Serum parameters, including lipid profile, troponin, creatinine, nitrotyrosine, malondialdehyde, nitrite, and brain natriuretic peptide, were measured. The electrocardiographic profile and renal vascular reactivity were evaluated. Cardiac histopathology and ventricular morphometry were performed, and the tissue enzymatic antioxidant system was investigated.

Results: A total of 37 compounds were detected in EEPC, including organic acids, phenolic acid derivatives, flavonoids, anthocyanins, and hydrolysable tannins (gallotannins and ellagitannins). EEPC treatment induced a cardiorenal protective response, prevented hemodynamic and functional alterations, and prevented ventricle remodeling. These effects were associated with the normalization of creatinine and brain natriuretic peptide levels and modulation of the tecidual antioxidant defense system.

Conclusion: The present study demonstrated that EEPC may prevent doxorubicin-induced heart failure by

Abbreviations: ACh, acetylcholine; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ANOVA, analysis of variance; ASE, accelerated solvent extraction; AST, aspartate aminotransferase; BNP, brain natriuretic peptide; CAT, catalase; DBP, diastolic blood pressure; DOX, doxorubicin; EEPC, ethanolic extract of *Plinia cauliflora*; ENAL, enalapril; GGT, gamma-glutamyl transpeptidase; HDL-C, high-density lipoprotein cholesterol; HHDP, hexahydroxydiphenyl; HR, heart rate; IV, interventricular septum; LA, left arm; LC-DAD-MS, liquid chromatography coupled to diode array detector and mass spectrometry; LL, left leg; LPO, lipid peroxidation; LV, left ventricle; MDA, malondialdehyde; MAP, mean arterial pressure; MF, molecular formula; NC, negative control group; NI, non-identified; NPS, sodium nitroprusside; NT, nitrotyrosine; Phe, phenylephrine; PSS, physiological saline solution; RA, right arm; RL, right leg; RT, retention time; RV, right ventricle; S.E.M., standard error of the mean; SBP, systolic blood pressure; SOD, superoxide dismutase; SPE, solid phase extraction; TC, total cholesterol

* Corresponding author. Laboratório de Eletrofisiologia e Farmacologia Cardiovascular (LEFaC), Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados, Rodovia Dourados-Itahum, km 12, P.O. Box 533, 79804-970, Dourados, MS, Brazil.

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modulating the antioxidant defense system, reducing reactive oxygen species-induced damage, preventing alterations of hemodynamic and endothelial function, and preventing damage to the cardiac structure. EEPC, especially at the highest dose tested, may be considered a cardioprotective coadjuvant to prevent doxorubicin-induced cardiotoxicity.

1. Introduction

Doxorubicin is utilized in various treatment regimens to control cancer, including breast cancer, lung cancer, gastric cancer, ovarian cancer, and Hodgkin's and non-Hodgkin's lymphomas (Thorn et al., 2011). However, the clinical use of doxorubicin is limited because of its systemic toxicity and the induction of cumulative and dose-dependent cardiotoxicity (Lee et al., 2017). The pathophysiology of doxorubicin-induced cardiomyopathy is predominantly determined by oxidative stress. Different mechanisms are involved in doxorubicin-induced cardiomyopathy, but the common outcome is cell death, such as apoptosis, necrosis, fibrosis, and autophagy (Renu et al., 2018).

Chemotherapy is associated with several side effects, such as nausea, hair loss, dizziness, and weakness. These effects impair the quality of life of patients and are linked to the abandonment of treatment (Chan and Ismail, 2014). Researchers have sought to prevent or attenuate such side effects, and studies support the possibility that antioxidant substances are able to achieve this purpose (Conklin, 2000; Palesh et al., 2018).

Oxidative stress that occurs during cancer treatment is mainly responsible for side effects that are observed in patients (Singh et al., 2018). Studies suggest that antioxidant substances, such as selenium and vitamins A, C, and E, are able to neutralize or scavenge reactive oxygen species (ROS) and prevent cell damage (Pan et al., 2011). Different natural compounds may also decrease or mitigate the adverse side effects of chemotherapy (Sak, 2012).

In Brazil, a native species that is popularly known as “jabuticaba” (*Plinia cauliflora* [Mart.] Kausel [Myrtaceae], synonymous with *Eugenia cauliflora* [Mart.] DC and *Myrciaria cauliflora* [Mart.] O. Berg) produces fruit that is widely consumed fresh or used for the production of beverages or jelly (Inada et al., 2015). In Brazilian folk medicine, this species is used to treat asthma, throat inflammation, and gastrointestinal and cardiovascular disturbances (Giraldi and Hanazaki, 2010). Its fruit has a purple peel that is rich in several phenolic compounds, including anthocyanins, and ellagic acid derivatives (Neves et al., 2018). Anthocyanins belong to the class of flavonoids and are pigments that are responsible for the coloration of many flowers and fruits. These compounds can act on the antioxidant system where they eliminate free radicals, thus reducing cellular damage that is caused by oxidative stress (Bowen-Forbes et al., 2010; Li et al., 2015). Pharmacological studies that have been performed over the last decade have reported antioxidant (de Souza et al., 2017), antiinflammatory (Donado-Pestana et al., 2018), hypolipidemic (Araújo et al., 2014), antifungal (Souza-Moreira et al., 2018), leishmanicidal (Silva et al., 2018), antibacterial (Silva et al., 2018), and cytotoxic (Silva et al., 2018) effects of different preparations of the fruit peel of *P. cauliflora*.

Despite the widespread use of *P. cauliflora* fruit peel in the production of different bioactive products, no studies have evaluated its long-term effects on the cardiovascular system. The present study evaluated the possible cardioprotective effects of a hydroethanolic extract of *Plinia cauliflora* (EEPC) in female rabbits in a model of doxorubicin-induced heart failure.

2. Material and Methods

2.1. Drugs

The following drugs, salts, and solutions were used: doxorubicin hydrochloride (Libbs-Pharmaceutical Industry, São Paulo, SP, Brazil), diazepam (Cristália, Itapira, SP, Brazil), ketamine hydrochloride (Syntec, São Paulo, SP, Brazil), and heparin (Hipolabor, Belo Horizonte, MG, Brazil). Phenylephrine, sodium nitroprusside, acetylcholine, NaCl, KCl, $CaCl_2$, $MgSO_4$, $NaHCO_3$, KH_2PO_4, dextrose, and ethylenediaminetetraacetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). All of the other reagents were obtained in analytical grade.

2.2. Phytochemical study

2.2.1. Plant material

Plinia cauliflora fruits were collected at Esperança Nova, Paraná, Brazil (latitude: −23.719864, longitude: −53.802104), in September 2017. A voucher specimen (no. 6337) was authenticated by Dr. Zefa Valdivina Pereira and deposited in the Herbarium of the Universidade Federal da Grande Dourados (UFGD). The fruit peels were manually removed and dried by forced air circulation for 5 days. Finally, the peels were pulverized in a knife mill and stored in plastic bags under refrigeration (2-8 °C) until use.

2.2.2. Extraction procedures by accelerated solvent extraction

The extract (EEPC) was prepared from fruit peels by accelerated solvent extraction (Dionex®) using ethanol-water (7:3, v/v). Nitrogen was used in the extraction procedures. The following parameters were applied and repeated three times: 125 °C temperature, 4 min static extraction time, 100% washing volume, 1500 psi pressure, and 60 s purge. The EEPC was concentrated by a rotary evaporator (Büchi R-3, Flawil, Switzerland) under reduced pressure and lyophilized (30% yield).

2.2.3. EEPC fractionation by solid phase extraction

The EEPC was fractionated by solid phase extraction (SPE) using a Strata C18-E reversed-phase SPE cartridge (55 µm, 70 A, Phenomenex®). For this purpose, 30 mg of EEPC extract was solubilized in 1 ml of Mili-Q water, centrifuged at 3000 rpm for 5 min, and then added to the previously prepared SPE cartridge. The fractions (elution with 12 ml of solvent) were obtained with the solvents 100% water (Fr–H_2O, 22 mg), 10% methanol (Fr-MeOH10, 0.4 mg), 25% methanol (Fr-MeOH25, 0.7 mg), 60% methanol (Fr-MeOH60, 0.5 mg), and 100% methanol (Fr-MeOH100, 0.2 mg). The fractions were concentrated by a rotary evaporator and dried by lyophilization.

2.2.4. Analyses of EEPC fractions by LC-DAD-MS

All of the fractions (Fr–H_2O, Fr-MeOH10, Fr-MeOH25, Fr-MeOH60, Fr-MeOH100) that were obtained by SPE were analyzed using an UFLC LC-20AD Prominence Shimadzu and a high-resolution mass spectrometer with an electrospray ionization source (MicrOTOF-Q III, Bruker Daltonics, Billerica, MA, USA). The chromatography column was a Kinetex C18 column (2.6 µm, 100 A, 150 mm × 2.1 mm, Phenomenex). All of the parameters that were used for LC and MS were the same as in Tolouei et al. (2019). The samples were prepared at a 1 mg/ml concentration in methanol-water (1:1, v/v), filtered through PTFE filters (Millex, 0.22 mm × 13 mm, Millipore), and injected at a volume of 1 µl. Acetonitrile (B) and water (A), to which 0.1% formic acid (v/v) was added, were applied as the mobile phase.

E-mail addresses: arquimedesjunior@ufgd.edu.br, arquimedesgasparotto@gmail.com (A. Gasparotto Junior).

2.3. Pharmacological study

2.3.1. Animals

Twenty-week-old female New Zealand rabbits (weighing 2.5–3.0 kg) were obtained from Universidade Federal do Paraná (UFPR, Brazil) and housed in the vivarium of UFGD under controlled temperature (20 °C ± 2 °C) and humidity (50% ± 10%) and a 12 h/12 h light/dark cycle with *ad libitum* access to food and water. All of the experimental procedures were approved by the Institutional Ethics Committee of UFGD (protocol no. 11/2018; approved March 16, 2018) and conducted in accordance with the Brazilian Legal Framework on the Scientific Use of Animals.

2.3.2. Experimental groups and induction of heart failure

Heart failure was induced according to the method of Gava et al. (2013), with a few modifications. First, doxorubicin (1.5 mg/kg) was administered intravenously once weekly for 5 weeks. After 1 week of doxorubicin administration, the animals were randomly assigned to five experimental groups (n = 6/group): (1) naive (the rabbits received placebo [distilled water] and were treated with vehicle [filtered water]), (2) negative control (the rabbits received doxorubicin and were treated with vehicle [filtered water]), (3) EEPC 75 (the rabbits received doxorubicin and were treated with 75 mg/kg EEPC), (4) EEPC 150 (the rabbits received doxorubicin and were treated with 150 mg/kg EEPC), and (5) ENAL 5 (the rabbits received doxorubicin and were treated with 5 mg/kg enalapril). EEPC was orally administered once daily for 4 weeks, starting from week 2 of doxorubicin administration. Mortality rates were monitored daily, and body weight was recorded weekly using an analytical scale. At the end of the experimental period (day 36), all of the animals were intramuscularly anesthetized with 10 mg/kg diazepam plus 115 mg/kg ketamine and underwent the following procedures.

2.3.3. Electrocardiography

After anesthesia, all of the rabbits were kept in the dorsal decubitus position. Four alligator clip electrodes (RL, RA, LL, and LA) were positioned in the folds of both elbows and both knees. The V1 electrode was positioned in the 4th intercostal space to the right of the sternum. The V2 electrode was positioned in the 4th intercostal space to the left of the sternum. The V3 electrode was positioned midway between V2

Table 1
Identification of the constituents from *P. cauliflora* extract by LC-DAD-MS.

Peak	RT (min)	Compound	MF*	UV (nm)	Negative mode (m/z)		Positive mode (m/z)	
					MS $[M-H]^-$	MS/MS	MS $[M+H]^+$	MS/MS
1	1.2	di-hexoside	$C_{12}H_{22}O_{11}$	–	341.1083	191	365.1034^{Na}	–
		Hexose	$C_6H_{12}O_6$		179.0563	–	203.0534^{Na}	–
		Quinic acid	$C_7H_{12}O_6$		191.0561	–	–	–
2	1.5	Citric acid	$C_6H_8O_7$	–	191.0181	–	–	–
3	2.5	Gallic acidst	$C_7H_6O_5$	270	169.0135	–	–	–
4	8.3	*O*-hexosyl quercetin	$C_{21}H_{20}O_{12}$	270, 352	463.0889	301, 255	487.0856^{Na}	303
5	9.2	NI	$C_{21}H_{20}O_{13}$	280	479.0847	299, 271, 255, 231, 191	481.0963	303
6	10.4	Di-*O*-galloyl hexoside	$C_{20}H_{20}O_{14}$	274	483.0796	313, 271, 169	485.0910	–
7	10.5	HHDP galloyl *O*-hexoside (corilagin isomer)	$C_{27}H_{22}O_{18}$	270	633.0764	463, 301, 275, 246, 169	–	–
8	10.7	Di-*O*-galloyl hexoside	$C_{20}H_{20}O_{14}$	280	483.0808	313, 271, 169	–	–
9	11.0	*O*-hexosyl delphinidin	$C_{21}H_{21}O_{12}^+$	276, 515	463.0904	–	465.1033	303
10	11.1	Tri-*O*-galloyl hexoside	$C_{27}H_{24}O_{18}$	280	635.0894	313, 295, 169	–	–
		di-HHDP galloyl *O*-hexoside (casuarinin isomer)	$C_{41}H_{28}O_{26}$	280	935.0787	301, 275, 169	937.0961	–
11	11.3	NI	$C_{27}H_{30}O_{16}$	280	609.1486	339, 309	611.1606	–
12	11.6	NI	$C_{19}H_{26}O_{13}$	270	461.1329	191	–	–
13	12.5	*O*-hexosyl cyanidin	$C_{21}H_{21}O_{11}^+$	279, 512	447.0958	284, 255, 239	449.1087	287
		HHDP di-galloyl *O*-hexoside	$C_{34}H_{26}O_{22}$		785.0847	419, 301, 275, 249, 169	787.0984	–
14	13.1	*O*-galloyl ellagic acid	$C_{21}H_{10}O_{13}$	270, 360	469.0075	301, 271, 228	471.0196	435, 407, 287
		Syringic acid	$C_9H_{10}O_{53}$		197.0461	167	199.0599	–
15	13.8	Tri-*O*-galloyl hexoside	$C_{27}H_{24}O_{18}$	275	635.00901	465, 313, 295, 169	637.1013	–
		di-HHDP *O*-hexoside	$C_{34}H_{24}O_{22}$		783.0718	301, 275, 169	785.0830	–
16	14.1	HHDP-galloyl *O*-hexoside	$C_{34}H_{26}O_{22}$	276	785.0874	301, 275, 246, 169	787.0987	–
17	14.7	*O*-hexosyl ellagic acid	$C_{20}H_{16}O_{13}$	255, 360	463.0549	301, 272, 257, 245	465.0665	303, 285, 275
18	15.1	di-HHDP-galloyl *O*-hexoside	$C_{41}H_{28}O_{26}$	275	935.0827	301, 275, 245, 217, 167	–	–
19	15.7	di-HHDP-galloyl *O*-hexoside (castalagin/vescalagin isomer)	$C_{41}H_{26}O_{26}$	272	933.0607	301, 275, 257	935.0760	–
20	16.0	di-HHDP-galloyl *O*-hexoside (castalagin/vescalagin isomer)	$C_{41}H_{26}O_{26}$	270	933.0645	301, 275, 257	935.0770	–
21	16.9	HHDP tri-galloyl *O*-hexoside	$C_{41}H_{30}O_{26}$	272	468.0459^{-2}	301, 275, 249	–	–
		di-HHDP *O*-hexoside	$C_{34}H_{24}O_{22}$	272	783.0663	451, 301, 275, 249	–	–
22	17.1	di-HHDP-galloyl *O*-hexoside (castalagin/vescalagin isomer)	$C_{41}H_{26}O_{26}$	270	466.0320^{-2}	301, 275, 257	935.0747	–
23	17.3	*O*-pentosyl ellagic acid	$C_{19}H_{14}O_{12}$	255, 358	433.0433	301	435.0577	303
24	17.6	Ellagic acidst	$C_{14}H_6O_8$	252, 364	300.9996	283, 257, 229, 173	303.0135	275, 257, 229, 201
25	17.9	*O*-deoxyhexosyl myricetin	$C_{21}H_{20}O_{12}$	265, 350	463.0885	316, 287, 271, 179	465.1036	319
26	18.3	*O*-deoxyhexosyl ellagic acid	$C_{20}H_{16}O_{12}$	265, 362	447.0576	300	449.0731	303, 275
27	20.2	NI	$C_{20}H_8O_{12}$	278	438.9970	339, 300, 285, 255	–	–
28	21.1	*O*-deoxyhexosyl quercetin	$C_{21}H_{20}O_{11}$	266, 350	447.0929	300, 271, 255, 243, 178,163	449.1097	303
29	25.4	*O*-cinnamoyl *O*-galloyl hexoside	$C_{22}H_{22}O_{11}$	279	461.1103	169, 161	–	
		Quercetin	$C_{15}H_{10}O_7$	265, 354	301.0345	179	303.0492	–

RT: retention time; MF: molecular formula; NI: non-identified; HHDP: hexahydroxydiphenyl; *considered the errors up 7 ppm and mSigma lower than 30; ^{Na}: $[M+Na]^+$; $^{-2}$: $[M-2H]^{-2}$;stconfirmed by the authentic standard.

and V4. The V4 electrode was positioned in the 5th intercostal space at the midclavicular line. The V5 electrode was positioned inside the axillary line at the same level as V4. The V6 electrode was positioned at the midaxillary line at the same level as V4 and V5. A small amount of 70% alcohol was applied to each interface of the electrodes for less interference and better electrical conductance. An acclimatization period of 5 min elapsed, and then cardiographic electrical waves were recorded for 5 min. Electrocardiography was recorded using an electrocardiography recorder (WinCardio, Micromed, Brasília, Brazil).

2.3.4. Blood pressure assessment

After electrocardiography, all of the rabbits subcutaneously received a bolus injection of heparin (50 IU). Tracheotomy was then performed to allow the animals to spontaneously breathe. The left carotid artery was then isolated, cannulated, and connected to a pressure transducer coupled to a PowerLab® recording system and an application program (Chart, v 4.1; all from ADI Instruments, Castle Hill, Australia), and systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) were recorded. Changes in SBP, DBP, and MAP were recorded for 20 min.

2.3.5. Biochemical parameters

After the arterial pressure measurements, blood samples were collected from the left carotid artery. Serum was obtained by centrifugation at 1500 × *g* for 10 min. Albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), indirect bilirubin, direct bilirubin, total bilirubin, creatinine, urea, uric acid, triglycerides, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), γ-glutamyl transpeptidase (GGT), sodium, and potassium levels were measured using an automated biochemical analyzer (Roche Cobas Integra 400 plus). Nitrotyrosine (NT), troponin, and brain natriuretic peptide (BNP) levels were measured using an enzyme-linked immunosorbent assay (MyBioSource, San Diego, CA, USA). Malondialdehyde (MDA) levels were measured using an MDA assay kit (Cayman Chemical, Ann Arbor, MI, USA). Plasma nitrite levels were determined by enzymatically reducing nitrate according to the technique that was described by Schmidt et al. (1989).

2.3.6. Vascular kidney reactivity

After blood collection and before euthanasia, the left kidneys were isolated and prepared for perfusion according to McGregor (1965). The kidneys were placed in a water-jacketed organ bath and perfused at 6 ml/min with PSS (119 mM NaCl, 4.7 mM KCl, 2.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11.1 mM dextrose, and 0.03 mM EDTA) at 37 °C and gassed with 95% O_2/5% CO_2. Changes in the perfusion pressure (PP; mmHg) were detected by a pressure transducer coupled to a PowerLab® recording system and an application program (Chart, v 4 0.1; all from ADI Instruments, Castle Hill, Australia). After equilibration for 30 min, the integrity of the preparation was checked by a bolus injection of 120 mmol KCl. Phenylephrine (Phe; 0.1, 0.3, and 1 nmol; 10–30 µl) and angiotensin II (Ang II; 0.1, 0.3, and 1 nmol; 10–30 µl) were then administered. After a new equilibration period (30 min), the kidneys were continuously perfused with PSS plus 3 µM Phe to induce a prolonged increase in PP. Under these conditions, vascular reactivity to sodium nitroprusside (SNP; 1, 3, and 10 pmol; 10–30 µl) and acetylcholine (ACh; 1, 3, and 10 pmol; 10–30 µl) was evaluated. An equilibration period of 15 min was allowed between each drug administration.

2.3.7. Relative weight and histopathological analysis of the heart and morphometry of the left ventricle

After euthanasia by an intravenous injection of 19.1% potassium chloride, the heart was removed, longitudinally sectioned, and cleaned. The relative weight of the heart was determined (WT% = absolute organ weight × 100/body weight). Part of the cardiac tissue was placed in 10% buffered formalin. The samples were then dehydrated in alcohol, cleared with xylene, and embedded in paraffin. Samples were sectioned (5 mm), stained with hematoxylin and eosin, and examined under a light microscope. Image data acquisition and analysis were performed using Motic Images Plus 2.0 software.

2.3.8. Cardiac and renal antioxidant defense system

After euthanasia, part of the cardiac and renal tissue was quickly collected and homogenized in potassium phosphate buffer (0.1 M, pH 6.5) in a 1:10 dilution. Superoxide dismutase (SOD) and catalase (CAT) activity and lipid hydroperoxide (LPO) levels were determined according to Gao et al. (1998), Beers and Sizer (1952), and Jiang et al. (1992), respectively. The results are expressed as U of SOD/g of tissue for SOD activity, mmol hydroperoxide/g of tissue for LPO levels, and mmol/min/g of tissue for CAT activity.

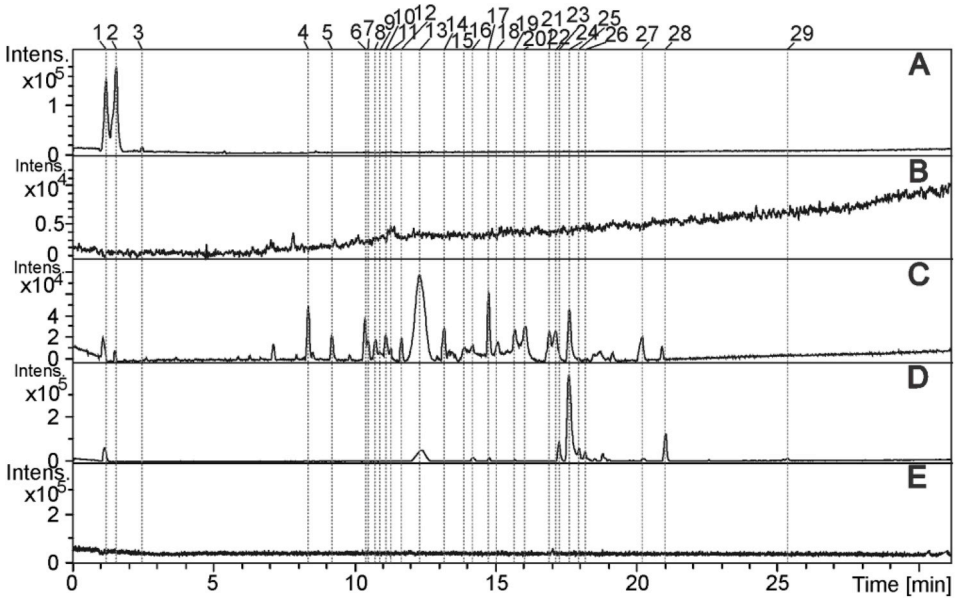

Fig. 1. Base peak chromatograms (negative ion mode) of the fractions Fr–H2O (A), Fr-MeOH10 (B), Fr-MeOH25 (C), Fr-MeOH60 (D) and Fr-MeOH100 (E) from Plinia cauliflora.

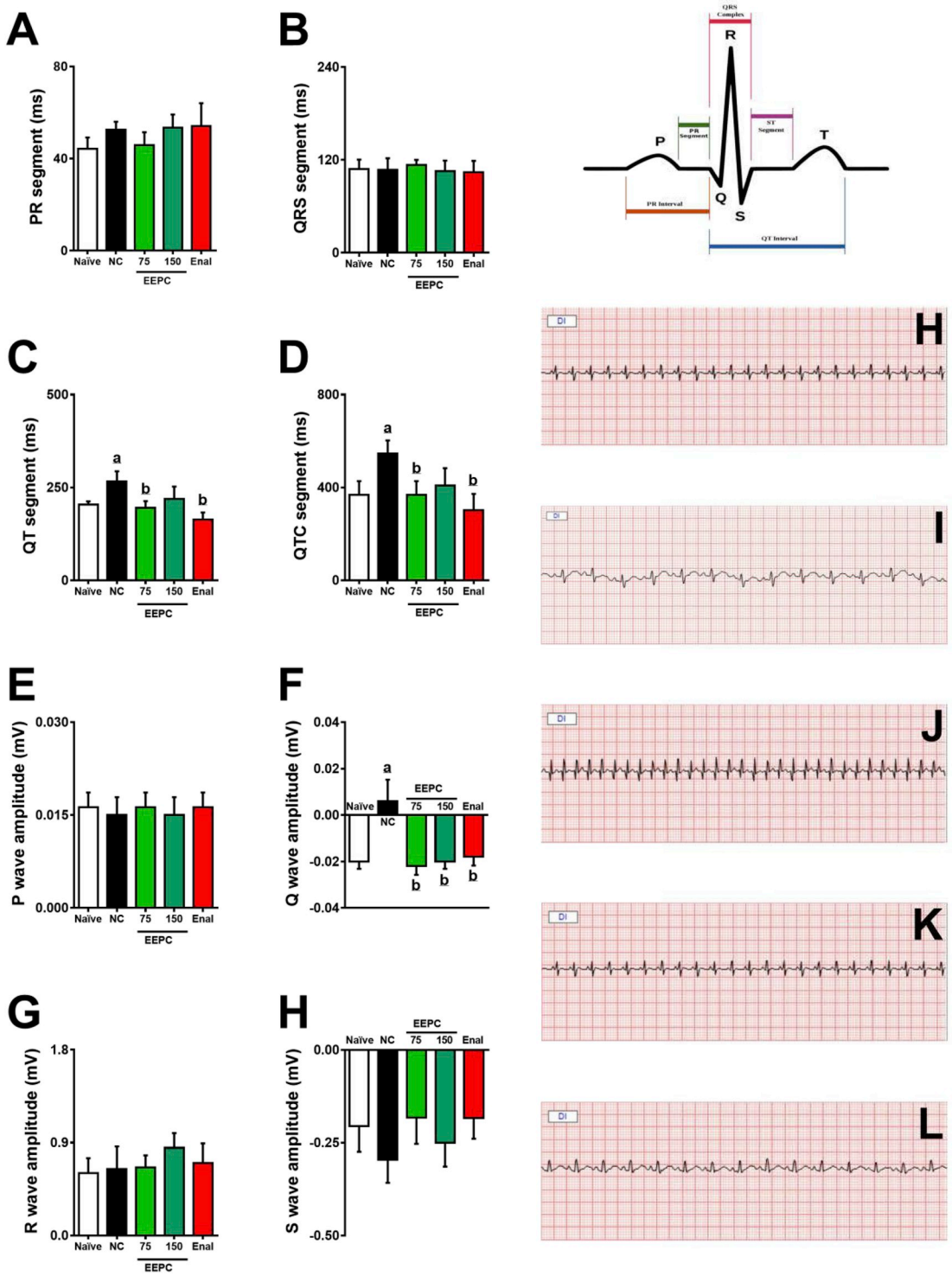

Fig. 2. Electrocardiographic quantitative data of naïve rabbits or with congestive heart failure treated with EEPC (75 and 150 mg/kg), ENAL (5 mg/kg), or vehicle (NC). PR (A), QRS (B), QT (C) and QTC-segments (D) and P (E), Q (F), R (G) and S-waves amplitude (H) are showed. Values are expressed as mean ± S.E.M. (n = 5-6) in comparison with naïve rabbits ($^{a}p < 0.05$) or NC ($^{b}p < 0.05$) using one-way ANOVA followed by Dunnett's test. Representative ECG tracing of naive, NC, EEPC 75, EEPC 150 and ENAL are shown in I, J, K, L and M respectively. ECG: electrocardiogram; ENAL: enalapril; EEPC: ethanol extract from *Plinia cauliflora*; NC: negative control.

2.4. Statistical analysis

Differences between groups were assessed using analysis of variance (ANOVA), followed by Dunnett's test. Values of $p < 0.05$ were considered statistically significant. The results are expressed as mean ± standard error of the mean (SEM). The graphs were drawn and the statistical analyses were performed using GraphPad Prism 6.0 software.

3. Results

3.1. Identification of the constituents of EEPC

The extract (EEPC) of *P. cauliflora* was fractionated by SPE, and the fractions were analyzed by LC-DAD-MS, which allowed the identification of compounds in *P. cauliflora* that have not been previously reported. Thirty-seven compounds in EEPC were detected, including phenolic acid derivatives, flavonoids, anthocyanins, and hydrolyzable tannins (gallotannins and ellagitannins), among others (Table 1). The compound identifications were based on UV, MS, and MS/MS and confirmed by an injection of authentic standards or comparisons with data in the literature.

Peaks 1 and 2 (Fig. 1) revealed ions at *m/z* 341.1083, 179.0563, 191.0561, and 191.0181 $[M\text{-}H]^-$, which were compatible with the molecular formulas $C_{12}H_{22}O_{11}$, $C_6H_{12}O_6$, $C_7H_{12}O_6$, and $C_6H_8O_7$. These compounds were putatively identified as di-hexoside, hexose, quinic acid, and citric acid, respectively. Compound 3 presented an intense band at the 270 nm wavelength in the UV spectrum and a deprotonated ion at *m/z* 169.0135 relative to $C_7H_6O_5$. This metabolite was identified as gallic acid and confirmed by a standard injection.

Compounds 6–8, 10, 15, 16, and 18–22 presented bands at ~270–280 nm in the UV spectra, similar to gallic acid chromophore (Neves et al., 2018). Compounds 6, 8, 10, and 15 also presented ions that are compatible with the molecular formulas $C_{20}H_{20}O_{14}$ and $C_{27}H_{24}O_{18}$ and fragment ions that resulted from the loss of galloyl (152 *u*) and water molecules (18 *u*), such as ions at *m/z* 313 and 169, that are compatible with *O*-galloyl dehydrohexoside (*m/z* 313) and gallic acid (*m/z* 169; Chen et al., 2018). These metabolites were identified as the gallotannins di-*O*-galloyl hexoside (6, 8) and tri-*O*-galloyl hexoside (10, 15). Peaks 7, 10, 15, 16, and 18–22 exhibited fragment ions at *m/z* 301, indicating the presence of hexahydroxydiphenyl groups (HHDP), which can be rearranged for ellagic acid in the mass spectrometer (Chen et al., 2018; Souza-Moreira et al., 2018). Thus, the ellagitannis HHDP galloyl *O*-hexoside (7, 16), di-HHDP galloyl *O*-hexoside (10, 18–20, 22), HHDP di-galloyl *O*-hexoside (13), di-HHDP *O*-hexoside (15, 21), and HHDP tri-galloyl *O*-hexoside (21) were identified in *P. cauliflora*.

Peaks 14, 17, 23–24, and 26 presented UV spectra that were compatible with ellagic acid (λ_{max} = ~265 and ~360 nm). An ion at *m/z* 300.9996 $[M\text{-}H]^-$ of compound 24 and fragment ions at *m/z* 283 $[M\text{-}H\text{-}H_2O]^-$, *m/z* 257 $[M\text{-}H\text{-}CO_2]^-$, and *m/z* 229 $[M\text{-}H\text{–}CO_2\text{–}CO]^-$ were observed, confirming the presence of ellagic acid (Reichert et al., 2018). Compounds 14, 17, 23, and 26 presented a product ion at *m/z* 301, indicating the presence of ellagic acid. The loss of 168, 162, 132, and 146 *u* suggested the presence of the substituents galloyl, hexosyl, pentosyl, and deoxyhexosyl (Renu et al., 2018; Chen et al., 2018; Liu and Seeram, 2018), respectively. Thus, compounds 14, 17, 23, and 26 were identified as *O*-galloyl ellagic acid, *O*-hexosyl ellagic acid, *O*-pentosyl ellagic acid, and *O*-deoxyhexosyl ellagic acid, respectively.

Compounds 4, 25, 28, and 29 presented UV spectra (λ_{max} = ~270 and ~350 nm) that are characteristic of flavonols. The loss of 162 and 146 *u* from compounds 4 and 25/28 confirmed the presence of the substituents hexosyl and deoxyhexosyl. Product ions at *m/z* 301/300 and 316 suggested the presence of the aglycones quercetin and myricetin (Neves et al., 2018). Compounds 9 and 13 presented two bands at ~275 and 515 nm in the UV spectra, suggesting the presence of anthocyanins. They exhibited the molecular formulas $C_{21}H_{21}O_{12}^+$ and $C_{21}H_{21}O_{11}^+$, calculated according to their accurate mass (*m/z* 465.1033 and 449.1087), and both compounds presented the loss of 162 *u*, indicating the presence of hexose and aglycones at *m/z* 303 and 287. These data are compatible with the anthocyanins *O*-hexosyl delphinidin and *O*-hexosyl cyanidin, which were previously reported to be contained in jabuticaba (Baldin et al., 2018; Neves et al., 2018).

3.2. Survival rates and body weight evolution

All six rats in the naive and EEPC-treated groups (75 and 150 mg/kg) had a survival rate of 100%. Survival rates for the negative control and ENAL-treated groups were both 83% ($n = 5$). We did not observe any significant differences in body weight between groups during the 6-week study (data not shown). The rabbits exhibited behavioral patterns that were typical for the species and genus, and food and water consumption was similar throughout the experiment.

3.3. Electrocardiography

Fig. 2A–H shows quantitative electrocardiography data for naive rabbits and rabbits with heart failure that were treated with EEPC (75 and 150 mg/kg), ENAL (5 mg/kg), and vehicle. We did not observe any significant changes in electrocardiographic characteristics of the PR and QRS segments or the amplitude of the P, R, and S waves in any of the experimental groups. However, electrocardiographic changes were observed in rabbits with heart failure that were treated only with vehicle. We observed significant prolongation of the QT and QTS

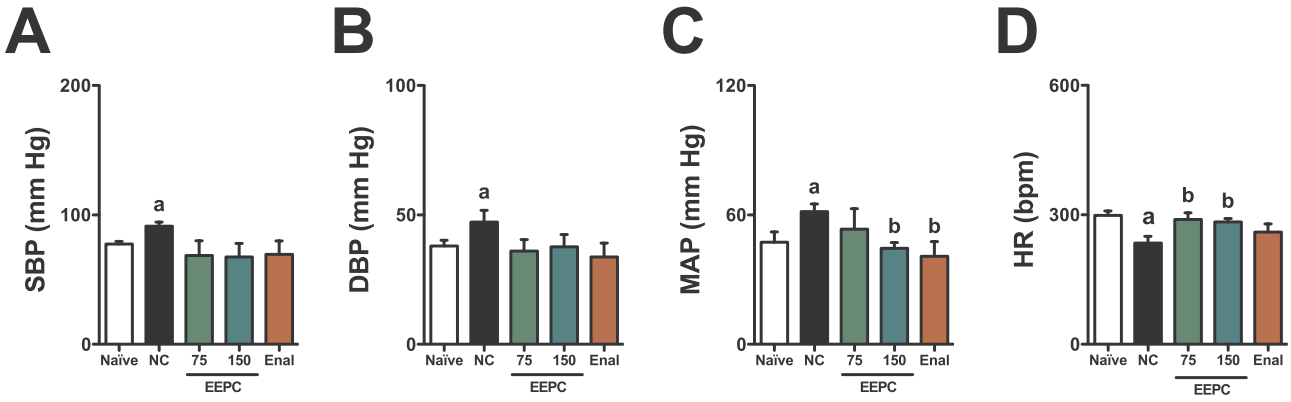

Fig. 3. Prolonged oral administration of EEPC obtained from *P. cauliflora* reverses changes in blood pressure and heart rate induced by heart failure. SBP (A), DBP (B), MAP (C), and HR (D) are showed. Values are expressed as mean ± S.E.M. (n = 5–6) in comparison with naïve rabbits ($^a p < 0.05$) or NC ($^b p < 0.05$) using one-way ANOVA followed by Dunnett's test. DBP: diastolic blood pressure; EEPC: ethanol extract from *Plinia cauliflora*; ENAL: enalapril; HR: heart rate; MAP: mean arterial pressure; NC: negative control; SBP: systolic blood pressure.

segments (Fig. 2C and D) and positive values for the Q-wave amplitude (Fig. 2F). All of these alterations were prevented by treatment with EEPC and ENAL. The values that were obtained for all of the parameters that were evaluated in animals that were treated with EEPC or ENAL were statistically similar to naive rabbits.

3.4. Effects on blood pressure

Basal SBP, DBP, MAP, and HR that were recorded after the 15-min stabilization period in naive animals were 77 ± 1.9 mmHg, 39 ± 2.2 mmHg, 47 ± 4.8 mmHg, and 298 ± 10 beats per minute (bpm), respectively. In animals with heart failure, SBP, DBP, MAP, and HR were 91 ± 3.0 mmHg, 47 ± 4.1 mmHg, 61 ± 3.5 mmHg, and 234 ± 15 bpm, respectively. Treatment with EEPC and ENAL prevented the cardiovascular changes that were observed in rabbits with heart failure, leading to SBP, DBP, MAP, and HR values that were similar to naive animals (Fig. 3A–D).

3.5. Effects on serum parameters

All of the rabbits with heart failure that were treated with vehicle alone exhibited significant increases in serum creatinine and BNP levels compared with naive animals. Treatment with EEPC and ENAL prevented the increase in serum creatinine levels, with values that were similar to naive rabbits (Table 2). Similarly, serum BNP levels significantly decreased in animals that received EEPC. Treatment with the higher dose of EEPC (150 mg/kg) and ENAL resulted in BNP levels that were similar to naive animals. All of the other serum parameters that were evaluated were not significantly different between groups (Table 2).

3.6. Effects on vascular kidney reactivity

In the negative control group, Ang II administration induced a vasoconstrictive effect in the kidneys, which was ~40% lower than in naive rabbits (Fig. 4B). Similarly, the vasodilatory response to ACh was significantly lower in the negative control group compared with naive animals (Fig. 4C). Prolonged treatment with EEPC (75 and 150 mg/kg) and ENAL reversed these changes, with values that were similar to naive rabbits. The effects of Phe and SNP were not different between groups (Fig. 4A, D).

3.7. Effects on cardiac morphology

The histological evaluations were consistent with cardiomyocyte hypertrophy. Compared with the naive group, the ultrastructure of the heart in the negative control group showed myofibril disarrangement, mitochondrial swelling, and a decrease in crista. Marked cardiac fibrosis was also detected. Treatment with EEPC (75 and 150 mg/kg) and ENAL attenuated ultrastructure disorganization in the doxorubicin-treated group, maintaining a cardiac structure that was similar to naive animals. The relative weight of the heart (WT%) and morphometric measures of the right ventricle (RV), left ventricle (LV), and interventricular septum (IV) are shown in Fig. 5A–D. We observed a significant increase in heart WT% (0.14% ± 0.002% *vs.* 0.18% ± 0.014%), RV posterior wall thickness (92 ± 2.6 µm *vs.* 118 ± 4.7 µm), LV posterior wall thickness (338 ± 7.5 µm *vs.* 403 ± 17 µm), and IV septum thickness (407 ± 5.4 mm *vs.* 463 ± 8.5 µm) in the negative control group compared with naive animals. Treatment with EEPC and ENAL reduced the thickness of the RV, LV, and IV septum in rabbits with heart failure, with values that were similar to naive animals.

3.8. Effects on redox status

Negative control animals exhibited significant increases in NT and MDA levels (~80% increase) compared with naive rabbits (Fig. 6A and B). Similarly, nitrite levels decreased from 41 ± 3.4 µM in the naive group to 20 ± 2.2 µM in the negative control group (Fig. 6C). Treatment with 75 and 150 mg/kg EEPC decreased NT and MDA levels to values that were similar to naive animals (naive: 2.74 ± 0.26 mol/L MDA, 0.015 ± 0.001 µmol/L NT; 75 mg/kg EEPC: 3.08 ± 0.33 mol/L MDA, 0.016 ± 0.001 µmol/L NT; 150 mg/kg EEPC: 2.61 ± 0.21 mol/L MDA, 0.014 ± 0.001 µmol/L NT). Similarly, nitrite levels were significantly increased by EEPC (75 and 150 mg/kg), with values that were close to naive animals (75 mg/kg EEPC: 35 ± 5.0 µM; 150 mg/kg EEPC: 40 ± 6.7 µM). The effects of ENAL treatment were not significantly different from EEPC treatment (Fig. 6A–C).

Table 2
Serum quantitative data of naïve rabbits or with congestive heart failure treated with EEPC, ENAL, or vehicle (NC).

Parameter	Naïve	NC	EEPC (75 mg/kg)	EEPC (150 mg/kg)	ENAL (5 mg/kg)
Albumin	3.01 ± 0.67	3.11 ± 0.88	2.80 ± 0.85	3.37 ± 0.72	2.93 ± 0.99
AST	29.1 ± 6.6	28.5 ± 9.0	33.2 ± 7.0	36.4 ± 8.4	31.9 ± 7.5
ALT	19.8 ± 6.1	21.1 ± 8.1	18.7 ± 6.7	19.4 ± 6.6	20.1 ± 7.4
I. bilirubin	0.04 ± 0.01	0.05 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
D. bilirubin	0.01 ± 0.008	0.01 ± 0.007	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.009
T. bilirubin	0.05 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
Creatinine	0.99 ± 0.19	2.95 ± 0.28[a]	1.42 ± 0.38[b]	1.54 ± 0.29[b]	1.22 ± 0.31[b]
Urea	29.1 ± 6.6	34.0 ± 8.6	38.4 ± 9.3	33.4 ± 2.5	31.1 ± 4.7
Uric acid	0.27 ± 0.09	0.23 ± 0.04	0.31 ± 0.05	0.38 ± 0.08	0.29 ± 0.07
Triglycerides	90.1 ± 16.3	87.3 ± 18.2	144.6 ± 35.4	103.2 ± 31.2	114.3 ± 22.3
TC	44.2 ± 12.1	46.4 ± 16.2	29.3 ± 9.2	39.1 ± 16.0	37.91 ± 11.3
HDL-C	22.5 ± 7.4	22.1 ± 7.1	14.4 ± 5.1	19.4 ± 7.1	20.2 ± 4.2
GGT	5.01 ± 0.98	5.31 ± 0.85	4.17 ± 1.07	6.11 ± 2.41	4.41 ± 1.97
ALP	23.1 ± 4.4	25.4 ± 9.3	19.5 ± 6.9	26.0 ± 9.1	22.6 ± 6.7
Na^+	105.2 ± 7.7	96.7 ± 9.1	103.7 ± 22.1	106.9 ± 11.7	98.9 ± 9.8
K^+	6.01 ± 1.71	6.23 ± 2.31	5.40 ± 1.71	6.52 ± 0.63	5.77 ± 1.01
Troponin	0.01 ± 0.005	0.01 ± 0.006	0.01 ± 0.003	0.01 ± 0.004	0.01 ± 0.005
BNP	85.11 ± 6.55	421.2 ± 11.[a]	201.2 ± 16.1[ab]	122.7 ± 16.9[bc]	143.32 ± 11.7[abc]

Values are expressed as mean ± S.E.M. (n = 5–6) in comparison with naïve rabbits ([a]$p < 0.05$), NC ([b]$p < 0.05$) or EEPC 75 mg/kg ([c]$p < 0.05$) using one-way ANOVA followed by Dunnett's test. ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BNP: brain natriuretic peptide; D: direct; ENAL: enalapril; EEPC: ethanol extract from *Plinia cauliflora*; GGT: gamma-glutamyl transpeptidase; HDL-C: high-density lipoprotein cholesterol; I: indirect; NC: negative control; T: total; TC: Total cholesterol.

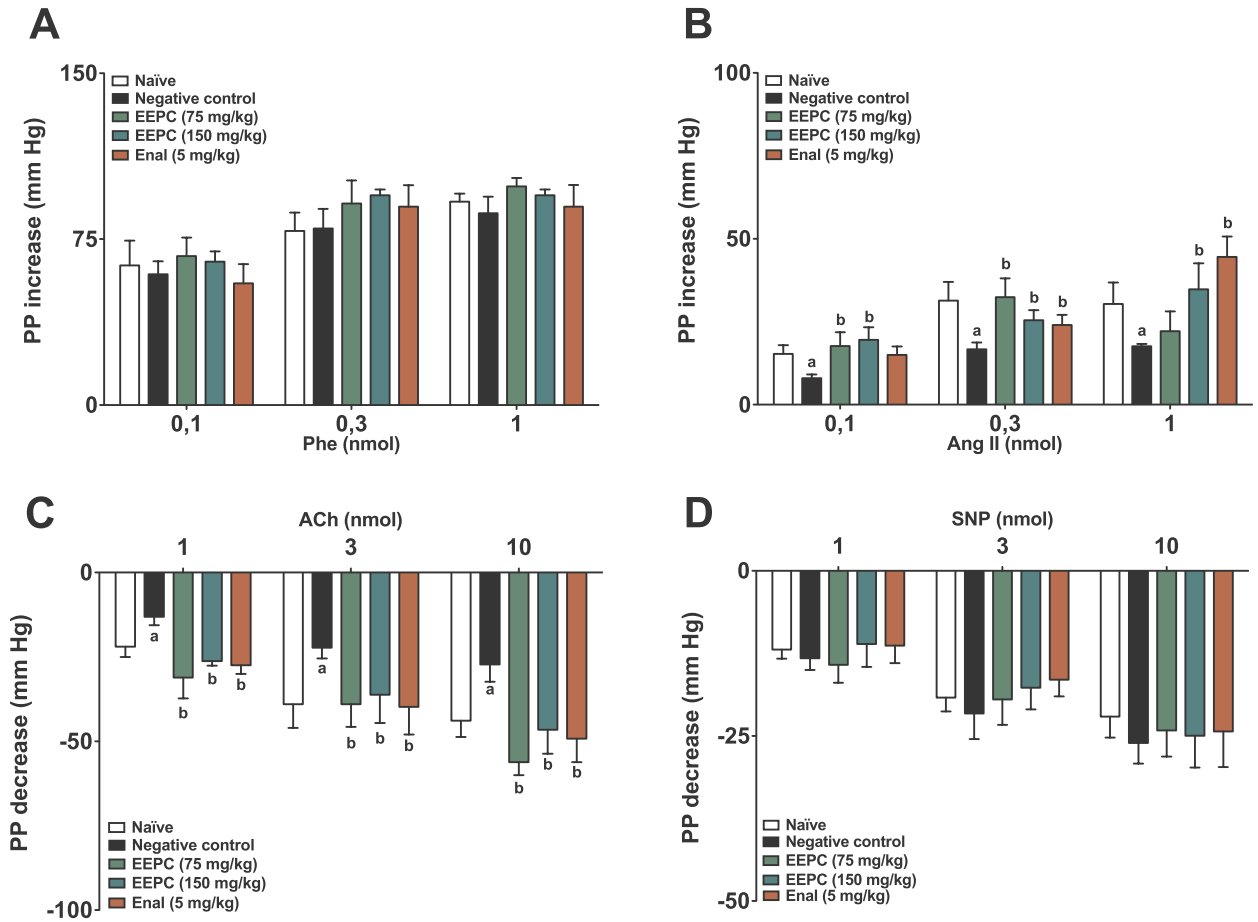

Fig. 4. Prolonged oral administration of EEPC obtained from *P. cauliflora* reverses changes in renal vascular reactivity induced by heart failure. Effects of phenylephrine (A), angiotensin II (B), acetylcholine (C), and sodium nitroprusside (D) are showed. Values are expressed as mean ± S.E.M. (n = 5–6) in comparison with naïve rabbits ($^{a}p < 0.05$) or NC ($^{b}p < 0.05$) using one-way ANOVA followed by Dunnett's test. ACh: acetylcholine; Ang II: angiotensin II; EEPC: ethanol extract from *Plinia cauliflora*; ENAL: enalapril; NC: negative control; Phe: phenylephrine; PP: perfusion pressure; SNP: sodium nitroprusside.

The antioxidant effects of EEPC on renal and cardiac tissue in rabbits that received doxorubicin are presented in Table 3. Cardiotoxicity that was induced by doxorubicin decreased SOD and CAT activity by ~50% in heart and kidney tissue compared with naive animals. Doxorubicin-induced heart failure increased LPO levels by more than 400% in renal and cardiac samples. Treatment with EEPC (especially at the highest dose) and ENAL reversed these changes in SOD, CAT, and LPO, leading to values that were similar to naive animals.

4. Discussion

The clinical utility of anthracycline agents is compromised by the

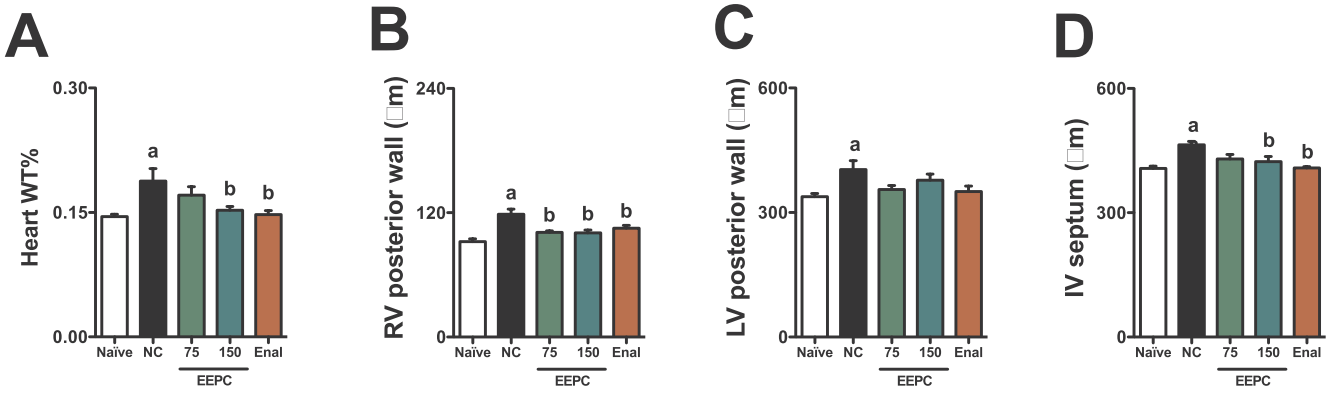

Fig. 5. EEPC treatment reduces heart histopathological changes induced by heart failure. WT% of the heart (A), and morphometric data of right ventricle posterior wall (B), left ventricle posterior wall (C), and interventricular septum (D) are shown. Representative cross-sections of the heart stained with hematoxylin-eosin are also presented (E). Values are expressed as mean ± S.E.M. (n = 5–6) in comparison with naïve rabbits ($^{a}p < 0.05$) or NC ($^{b}p < 0.05$) using one-way ANOVA followed by Dunnett's test. EEPC: ethanol extract from *Plinia cauliflora*; ENAL: enalapril; IV: interventricular; LV: left ventricle; NC: negative control; RV: right ventricle; WT%: relative weight.

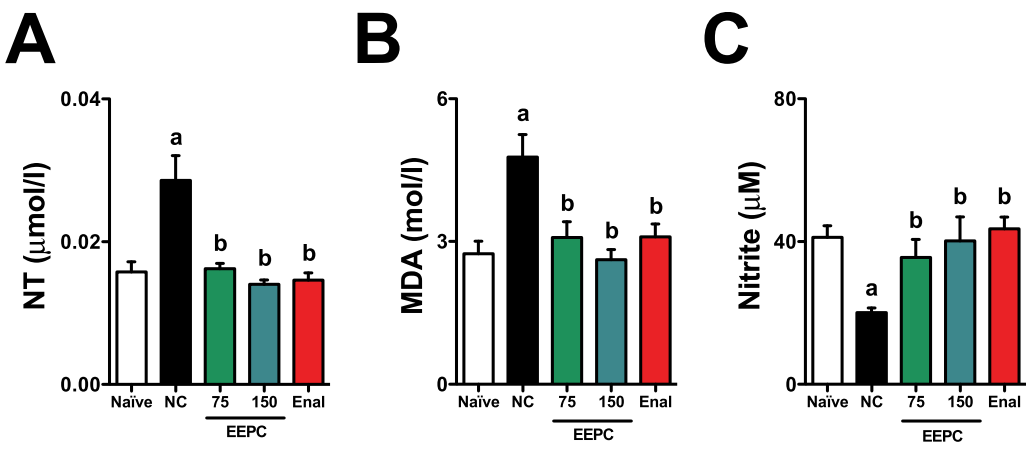

Fig. 6. Prolonged oral administration of EEPC obtained from *P. cauliflora* reverses changes in serum levels of nitrotyrosine (NT), malondialdehyde (MDA) and nitrite. NT (A), MDA (B), and nitrite levels (C) are showed. Values are expressed as mean ± S.E.M. ($n = 5–6$) in comparison with naïve rabbits ($^{a}p < 0.05$) or NC ($^{b}p < 0.05$) using one-way ANOVA followed by Dunnett's test. EEPC: ethanol extract from *Plinia cauliflora*; ENAL: enalapril; NC: negative control.

Table 3
Quantitative data of the antioxidant tissue defense system of naïve rabbits or with congestive heart failure treated with EEPC, ENAL, or vehicle (NC).

Parameter	Naïve	NC	EEPC (75 mg/kg)	EEPC (150 mg/kg)	ENAL (5 mg/kg)
Heart					
SOD	40 ± 4.2	17 ± 0.9[a]	43 ± 1.7[b]	63 ± 6.6[ab]	35 ± 2.1[bd]
CAT	4.5 ± 0.5	2.4 ± 0.5[a]	2.1 ± 0.3[a]	4.1 ± 0.8[b]	3.3 ± 2.2
LPO	320 ± 16	1702 ± 334[a]	739 ± 136[ab]	520 ± 139[b]	821 ± 73[ab]
Kidney					
SOD	30 ± 5.2	11 ± 0.4[a]	28 ± 2.3[b]	25 ± 3.1[b]	24 ± 2.9[b]
CAT	7.0 ± 0.8	4.0 ± 0.8[a]	6.8 ± 0.3[b]	6.7 ± 0.7[b]	5.2 ± 1.3
LPO	312 ± 55	1672 ± 282[a]	433 ± 68[b]	1013 ± 85[abc]	1123 ± 70[abc]

Values are expressed as mean ± S.E.M. (n = 5–6) in comparison with naïve rabbits ($^{a}p < 0.05$), NC ($^{b}p < 0.05$), EEPC 75 mg/kg ($^{c}p < 0.05$) or EEPC 150 mg/kg ($^{d}p < 0.05$) using one-way ANOVA followed by Dunnett's test. CAT: catalase (mmol/min/g tissue); ENAL: enalapril; EEPC: ethanol extract from *Plinia cauliflora*; LPO: lipid peroxidation (nmol hydroperoxides/g tissue); SOD: superoxide dismutase (Unit of SOD/g tissue).

risk of cardiotoxicity. An average of 10% of patients who are treated with doxorubicin or its derivatives develop cardiac complications up to 10 years after the end of chemotherapy. Oxidative stress has been shown to be the primary cause of cardiotoxicity. However, interventions that seek to reduce oxidative stress have not successfully reduced the incidence of cardiotoxicity in patients who are treated with doxorubicin (Octavia et al., 2012). New insights into the cardiomyocyte response to oxidative stress demonstrate that underlying differences between *in vitro* and *in vivo* toxicity may modulate the response to superoxide radicals and related compounds (Cappetta et al., 2017). This has led to potentially new uses for existing drugs and new avenues of exploration to discover better pharmacotherapies and interventions to prevent cardiotoxicity. However, much work is still needed to validate the clinical utility of these new approaches and proposed mechanisms.

One of the first alterations that may occur after doxorubicin administration involves changes in cardiac potassium channels. Humans have at least four major potassium channels with different densities and time- and voltage-dependent properties that contribute to ventricular repolarization in the myocardium and the maintenance of sinus rhythm (Surawicz, 1992). Doxorubicin downregulates these channels through caspase activation, thus inducing changes in cardiac electrical activity (Strigli et al., 2018). The administration of doxorubicin in female rabbits resulted in prolongation of the QT segment and a pathological Q wave. Alterations of the QT segment and Q wave may be attributable to variations in repolarization in different regions of the myocardium (Tse, 2016). In hypertrophic hearts and in conditions of dilated cardiomyopathy, these alterations may be attributable to areas of slow conductance, which is an important substrate for ventricular arrhythmias (Zhang et al., 2014). Alterations of gap junction intercellular communication, which is responsible for electrical contact between cardiomyocytes, are another mechanism that contributes to these changes. Connexins are gap junction proteins. Connexin-43 is mainly expressed in the heart. In healthy hearts, this protein is located in the intercalated disc. A decrease in its density is observed in remodeled hearts, and this mechanism may lead to prolongation of the QT interval and arrhythmias (Coronel et al., 2013).

In addition to electrocardiographic alterations, we observed interesting hemodynamic and biochemical changes in rabbits that were treated with doxorubicin. Significant reductions of HR, followed by a significant increase in blood pressure, were also observed. These alterations may be attributable to direct changes in ventricular repolarization or a compensatory response to higher blood pressure that results from doxorubicin-induced cardiomyopathy (i.e., cardiac overload; Alp et al., 2003). With regard to biochemical markers, we observed significant changes in the serum levels of BNP and creatinine. Brain natriuretic peptide is a hormone that is released by the ventricles when the heart is subjected to either chronic or acute damage. Cardiotoxicity that is caused by doxorubicin is attributable to free radical-mediated damage that causes the liberation of BNP in the bloodstream (Doroshow, 1983). The levels of BNP are proportional to cardiac damage (Silver et al., 2004). Thus, changes in the levels of BNP reflected significant changes in cardiac function. Thus, we also investigated the influence of cardiac output on renal function because creatinine levels also increased.

Renal dysfunction is common in patients with heart failure, with a clearly negative impact on prognosis (Ter Maaten et al., 2017). High serum creatinine levels reflect changes in renal clearance that are partially caused by the destruction of kidney cells by doxorubicin or indirectly caused by the progressive reduction of cardiac output that is caused by heart failure (Lahoti et al., 2012). One of the primary causes of low glomerular filtration rate may be related to changes in renal arteriolar reactivity. The vascular endothelium produces several vasoactive substances, either constrictors or dilators. When damage to the endothelium occurs, physiological responses may be observed that differ from an intact endothelium (Renu et al., 2018). The Ach-induced decrease in endothelium-dependent responsiveness shows that

doxorubicin-induced heart failure leads to direct or indirect damage to the vascular endothelium (Alp et al., 2003). Furthermore, the Ang II-induced decrease in renal reactivity suggests a lower vasoconstrictor response in the renal efferent arteriole. These effects may reduce the glomerular filtration rate and directly affect renal function (Feher, 2012).

As a final response to biochemical and hemodynamic changes, structural alterations occur, reflected by cardiovascular remodeling (Schaper et al., 2002). The main cause of cardiac remodeling consists of ventricular dysfunction, which starts with genetic alterations in response to cardiac injury. Subsequently, molecular and cellular changes occur, resulting in the progressive loss of ventricular function (Heusch et al., 2014). Cardiac remodeling is mainly related to alterations of geometry, including wall thickness, cavity diameter, and configuration of the LV (Cohn et al., 2000). In the present study, we observed several structural changes in the myocardium in doxorubicin-treated rabbits. These changes were accompanied by significant increases in serum and tissue oxidative stress. Doxorubicin-induced heart failure is associated with an increase in oxidative stress (Kim et al., 2006). We observed increases in serum NT and MDA levels in doxorubicin-treated rabbits, reflecting increases in protein oxidation and lipid peroxidation. Moreover, serum nitrite levels decreased, indicating a significant decrease in the bioavailability of nitric oxide. Furthermore, we evaluated redox status in renal and cardiac tissues and found that rabbits with heart failure exhibited decreases in SOD and CAT activity and an increase in LPO levels (400% increase) compared with the naive group.

We found that treatment with EEPC exerted important cardioprotective effects against doxorubicin-induced heart failure. Prolonged treatment with EEPC normalized electrocardiographic, hemodynamic, biochemical, and cardiac structural changes, in which EEPC-treated animals presented cardiovascular parameters that were similar to naive animals and animals that were treated with ENAL. Unknown are the pharmacological mechanisms by which EEPC exerts its beneficial effects. One possibility is that its activity may be related to the presence of several antioxidant compounds that were phytochemically identified (Bubols et al., 2013; Kaiserová et al., 2007). One limitation of the present study is that we did not identify which of the secondary metabolites may be responsible for the beneficial effects of EEPC. Its antioxidant and cardioprotective effects be attributable to different secondary metabolites that act in concert and possibly synergistically. Another limitation of the present study was that we did not evaluate possible synergistic or additive effects of ENAL and EEPC. Future studies should investigate whether the cardioprotective effects of EEPC could be improved by the concomitant administration of a classic cardioprotective drug.

5. Conclusion

The present study found that EEPC prevented doxorubicin-induced heart failure by modulating the antioxidant defense system, reducing ROS-induced damage, preventing alterations of hemodynamic and endothelial function, and preventing damage to the cardiac structure. EEPC, especially at the highest dose, has treatment potential as a cardioprotective coadjuvant to prevent doxorubicin-induced cardiotoxicity.

Author's contributions

All authors participated in the design, interpretation of the studies, analysis of the data and review of the manuscript; PVMR, RACP, LPG, AOS, BRL, CCFSM and ELBL conducted the experiments; DBS and SRN were involved with the preparation and chemical analysis of extract; AGJ and RACP were responsible for data discussion and manuscript correction. AGJ was the senior researcher responsible for this work. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2019.112042.

Conflicts of interest

Authors declare no conflicts of interest.

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6. CONCLUSÕES

A partir do conjunto de dados apresentados no presente estudo podemos concluir que os melhores processos extrativos em ordem de concentração de conteúdo fenólico foram o extrato hidroacetônico, hidroetanólico, etanólico e aquoso de *Plinia cauliflora* (Mart.) Kausel respectivamente. Entretanto, o extrato hidroetanólico de *P. cauliflora* (EEPC) foi o escolhido para o prosseguimento dos testes, pois esta composição de solventes apresenta menor toxicidade quando em comparação com as outras.

O EEPC se mostrou um efetivo agente cardioprotetor frente à cardiotoxicidade induzida pela doxorrubicina, pois foi capaz de reverter parcialmente as alterações eletrocardiográficas observadas após o tratamento por 6 semanas.

De fato, o EEPC apresentou importante atividade antioxidante sistêmica, uma vez que aumentou o aporte enzimático de defesas antioxidantes tecidual, tanto cardíaco como renal, e reduziu os níveis de dano oxidativo produzido pela lipoperoxidação.

Ademais preveniu as alterações cardiorrenais bioquímicas, hemodinâmicas e ventriculares. Além disso, sua utilização em uma ampla faixa de dose, não provocou efeitos farmacodinâmicos sistêmicos adversos.

7. ANEXOS

PARECER DE APROVAÇÃO DO COMITÊ DE ÉTICA

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, 18 de Outubro de 2019.

CERTIFICADO

Certificamos que a proposta intitulada ***"Avaliação da segurança farmacológica e eficácia de Plinia cauliflora (Mart.) Kausel em coelhos submetidos à um modelo de insuficiência cardíaca induzida por doxorrubicina"***, registrada sob o protocolo de nº 22/2019, sob a responsabilidade de *Arquimedes Gasparotto Junior* – 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 27/09/2019.

Finalidade	*( ) Ensino (X) Pesquisa Científica*
Vigência da autorização	*01/11/2019 a 01/03/2020*
Espécie/linhagem/raça	*Oryctolagus cuniculus – Coelhos Nova Zelândia*
Nº de animais	48
Peso/idade	*20 semanas / 2,5 - 3,0 kg*
Sexo	18 machos e 30 fêmeas
Origem	Biotério Central da UFPR – Curitiba/PR

Melissa Negrão Sepulvida

Melissa Negrão Sepulvida
Coordenadora CEUA