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Tese

AVALIAÇÃO FARMACOLÓGICA DE FRUTOS NATIVOS EM MODELO ANIMAL DE SÍNDROME METABÓLICA

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Bioquímica e Bioprospecção da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutora em Ciências (Bioquímica e Bioprospecção).

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Tese aprovada, como requisito parcial, para obtenção do grau de Doutora em Bioquímica e Bioprospecção, Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas.

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“Descobrir consiste em olhar para o que todo mundo está vendo e pensar uma coisa diferente”.

(Roger Von Oech)

Resumo

OLIVEIRA, Pathise Souto. **Avaliação farmacológica de frutos nativos em modelo animal de síndrome metabólica.** 2018. 161f. Tese (Doutorado)- Programa de Pós-Graduação em Bioquímica e Bioprospecção. Universidade Federal de Pelotas, Pelotas, 2018.

A síndrome metabólica é caracterizada por uma série de fatores de risco cardiovascular, como hiperglicemia, resistência à insulina, obesidade visceral e dislipidemia. Tendo em vista a complexidade dessa síndrome, a heterogeneidade molecular presente em extratos naturais os creditam como importantes agentes na terapêutica dessa desordem. Dessa forma, o presente estudo objetivou caracterizar fitoquimicamente os extratos hidroalcoólicos de frutos vermelhos de *Psidium cattleianum* e *Eugenia uniflora* e avaliar seus efeitos sobre parâmetros metabólicos, comportamentais, neuroquímicos, inflamatórios e tromboregulatórios em cérebro e/ou sangue de ratos Wistar machos de 21 dias submetidos ao modelo de síndrome metabólica induzida pelo consumo de dieta hiperpalatável (DHP). Os animais foram tratados durante 150 dias e divididos em 6 grupos experimentais: dieta padrão (DP) e água por gavagem, DP e extrato de *P. cattleianum* ou *E. uniflora*, DHP e água por gavagem, DHP e extrato de *P. cattleianum* ou *E. uniflora.* A dose dos extratos administrada foi de 200 mg/kg/dia por gavagem. A análise por cromatografia liquída acoplada à espectrometria de massa de alta eficiência (LC/MS) mostrou a presença de nove antocianinas no extrato de *E. uniflora* e revelou a cianidina-3-*O*-glicosídeo como a única antocianina presente no extrato de *P. cattleianum.* Os animais expostos a DHP apresentaram intolerância à glicose, aumento de peso e gordura visceral, níveis séricos elevados de glicose, triglicerídeos, colesterol total, colesterol LDL e interleucina-6, bem como aumento no tempo de imobilidade no teste do nado forçado. Essas alterações foram prevenidas pelos extratos testados. Adicionalmente, esses extratos diminuíram a peroxidação lipídica e preveniram a redução na atividade das enzimas superóxido dismutase e catalase em córtex pré-frontal, hipocampo e estriado. Ainda, ambos os extratos testados preveniram o aumento da atividade da acetilcolinesterase cortical causada pela DHP. O extrato de *P. cattleianum* preveniu o aumento nos níveis de nitrito, espécies reativas de oxigênio e restaurou a atividade da Ca^{2+}-ATPase nas estruturas cerebrais testadas, bem como a atividade da Na^{+}, K^{+}-ATPase no córtex pré-frontal e no hipocampo. O tratamento com os extratos de *P. cattleianum* e *E. uniflora* preveniu a redução na atividade da NTPDase nos linfócitos e plaquetas e da 5'-nucleotidase nas plaquetas causado pela DHP. Adicionalmente, a DHP aumentou a atividade da adenosina desaminase em linfócitos e soro. No entanto, o tratamento com extrato de *E. uniflora* preveniu esse aumento. Ademais, ambos os extratos impediram o aumento da atividade da acetilcolinesterase nos linfócitos e da butirilcolinesterase no soro induzido pela DHP. Em conclusão, os extratos de *E. uniflora e P. cattleianum* demonstraram efeitos anti-hiperglicêmicos, anti-hiperlipidêmicos, anti-inflamatórios, antitrombóticos, bem como propriedades neuroprotetoras, demonstrando ser potenciais agentes terapêuticos na prevenção de complicações associadas à síndrome metabólica.

Palavras chave: Síndrome metabólica. Dieta hiperpalatável. *E. uniflora. P. cattleianum.* Neuroproteção.

Abstract

OLIVEIRA, Pathise Souto. **Pharmacological evaluation of native fruits in animal model of metabolic syndrome.** 2018. 161f. Thesis (Doctorate)- Programa de Pós-Graduação em Bioquímica e Bioprospecção. Universidade Federal de Pelotas, Pelotas, 2018.

Metabolic syndrome is characterized by a variety of cardiovascular risk factors, such as hyperglycemia, insulin resistance, visceral obesity and dyslipidemia. Considering the complexity of this syndrome, the molecular heterogeneity presents in natural extracts credit them as important agents in the therapy of this disorder. Thus, the aim of the present study was to characterize phytochemically the hydroalcoholic extracts of red fruits (*Psidium* cattleianum and *Eugenia uniflora*) and to evaluate their effects on metabolic, behavioral, neurochemical, inflammatory and thromboregulatory parameters in the brain and/or blood of 21-day-old male Wistar rats submitted to the model of metabolic syndrome induced by consumption of highly palatable diet (HPD). The animals were treated for 150 days and divided into 6 experimental groups: standard chow (SC) and water by gavage, SC and extract of *P. cattleianum* or *E. uniflora*, HDP and water by gavage, HPD and extract of *P. cattleianum* or *E uniflora*. The dose of the extracts administered was 200 mg/kg/day by gavage. The liquid chromatography coupled to high efficiency mass spectrometry (LC/MS) analysis showed the presence of nine anthocyanins in the *E. uniflora* extract and revealed the cyanidin-3-*O*-glycoside as the only anthocyanin present in the extract of *P. cattleianum.* Animals exposed to HPD showed glucose intolerance, visceral fat and weight gain, elevated serum glucose, triglycerides, total cholesterol, LDL cholesterol and interleukin-6, as well as increased immobility time in the forced swimming test. These changes were prevented by the extracts tested. Additionally, these extracts decreased lipid peroxidation and prevented the reduction of superoxide dismutase and catalase activities in the prefrontal cortex, hippocampus and striatum. Furthermore, both extracts prevented the increase of cortical acetylcholinesterase activity caused by HPD. The extract of *P. cattleianum* prevented the increase in nitrite levels, reactive oxygen species and restored Ca^{2+}-ATPase activity in the brain structures tested, as well as Na^{+}, K^{+}-ATPase activity in the prefrontal cortex and hippocampus. Treatment with extracts of *P. cattleianum* and *E. uniflora* prevented the reduction in NTPDase activity in lymphocytes and platelets and the 5'-nucleotidase in platelets caused by DHP. Moreover, HPD increased the activity of adenosine deaminase in lymphocytes and serum. However, treatment with *E. uniflora* extract prevented this increase. In addition, both extracts prevented the increase of acetylcholinesterase activity in lymphocytes and butyrylcholinesterase in serum induced by HPD. In conclusion, the extracts of *E. uniflora* and *P. cattleianum* demonstrated antihyperglycemic, antihyperlipidemic, anti-inflammatory, antithrombotic effects, as well as neuroprotective properties, proving to be potential therapeutic agents in the prevention of complications associated to the metabolic syndrome.

Key words: Metabolic syndrome. Highly palatable diet. *E. uniflora. P. cattleianum.* Neuroprotection.

Lista de Figuras

Lista de abreviaturas e fórmulas químicas

ACh – Acetilcolina

AChE – Acetilcolinesterase

ADA – Adenosina desaminase

ADP – Adenosina difosfato

AMP – Adenosina monofosfato

ATP – Adenosina trifosfato

BuChE – Butirilcolinesterase

CAT – Catalase

ERO – Espécies reativas de oxigênio

DHP – Dieta hiperpalatável

DP – Dieta padrão

DM2 – Diabetes Mellitus tipo 2

GPx – Glutationa peroxidase

H_2O_2 – Peróxido de hidrogênio

HClO – Ácido hipocloroso

IL-6 – Interleucina-6

IL-1β – Interleucina-1 beta

IMC – Índice de massa corporal

NF-κB– Fator Nuclear Kappa B

NTPDase – Nucleosídeo trifosfato difosfoidrolase

$O_2^{\bullet-}$ – Ânion superóxido

$OH^{\bullet}$ – Radical hidroxila

OMS – Organização Mundial da Saúde

P2X – Receptor purinérgico

P2Y – Receptor purinérgico

PAI-1 – Inibidor-1 do ativador de plasminogênio

PCR – Proteína-C reativa

RI – Resistência à insulina

sICAM – Molécula de adesão intracelular solúvel

SM – Síndrome metabólica

SNC – Sistema nervoso central

SOD – Superoxido dismutase

TG – Triglicerídeos

TNFα – Fator de necrose tumoral alfa

VCAM – Molécula de adesão celular vascular

Sumário

1. Introdução

Nas últimas décadas, o Brasil mudou o seu cenário epidemiológico, no qual as doenças crônico-degenerativas, como as doenças cardiovasculares, síndrome metabólica (SM) e diabetes mellitus tipo 2 (DM2), assumem importância cada vez maior como causa de morbimortalidade (SBC, 2005). Além disso, estudos epidemiológicos revelam prevalências elevadas da SM na população brasileira e, dependendo do critério utilizado e das características da população estudada, as taxas variam entre 30 a 60% dos índivíduos analisados (LEITÃO & MARTINS, 2012; SAAD et al., 2014; ZORASKI et al., 2017; CALIXTO et al., 2018). Ainda, a SM tem apresentado prevalência mundial cresecente, a qual pode estar relacionada com o aumento da obesidade, sedentarismo, alimentação inadequada, bem como com o processo de envelhecimento (LEITÃO & MARTINS, 2012; LIN & ECKEL, 2014; PALEY & JOHNSON, 2018). É importante destacar também, que aproximadamente 50% dos adultos em todo o mundo devem ser obesos até 2030 (SBC, 2005; PALEY & JOHNSON, 2018).

A SM é definida como um conjunto de fatores de risco cardiovascular que incluem a hiperglicemia, hipertensão, dislipidemia, obesidade visceral e resistência à insulina (RI) (MAYUKO et al., 2013; FRANKENBERG et al., 2017; PALEY & JOHNSON, 2018). Os sintomas da SM pioram com o consumo de alimentos de alta densidade energética como os alimentos altamente palatáveis e a inatividade física (SOUZA et al., 2007).

A RI é considerada um dos mecanismos patofisiológicos mais aceitos que caracterizam a SM, pois, juntamente com a intolerância à glicose, está presente na maioria das pessoas que apresentam essa síndrome. No entanto, a obesidade que envolve grande parte da população tem sido um dos fatores mais importantes relacionados com o aumento da prevalência da SM (DE CARVALHO VIDIGAL et al., 2013; LIN & ECKEL, 2014; FRANKENBERG et al., 2017). Estudos recentes sugerem que o acúmulo de tecido adiposo na região do abdômen precede o desenvolvimento de outros componentes da síndrome e que a redução de peso pode ser o melhor meio de prevenir a doença (LIN & ECKEL, 2014; PALEY & JOHNSON, 2018). O aumento da gordura visceral desempenha um papel importante na agressão ao miocárdio por meio da produção de citocinas pró-inflamatórias, como o fator de necrose tumoral (TNF-α),

interleucina-1 beta (IL-1β), interleucina-6 (IL-6), proteína C reativa (PCR), inibidor-1 do ativador do plasminogênio (PAI-1), dentre outras (WISSE et al., 2004; BULLO et al., 2007; LIN & ECKEL, 2014; SLOP et al., 2017; PALEY & JOHNSON, 2018).

Adicionalmente, o aumento da ingestão energética pode induzir o estresse oxidativo e aumentar a produção de espécies reativas de oxigênio (ERO) (SAVINI et al., 2013; SLOP et al., 2017; JHA et al., 2017; PALEY & JOHNSON, 2018). Ainda, o aumento de ERO e de moléculas pró-inflamatórias pode estar diretamente relacionado com o desenvolvimento da obesidade, aterosclerose, DM2, RI, doenças neurológicas, distúrbios cognitivos e neuropsiquiátricos (MURDOLO et al., 2013; GANCHEVA et al., 2017; BETTIO et al., 2017; JHA et al., 2017; DE LA MONTE et al., 2018; AMBRÓSIO et al., 2018). Há diversos fatores que interferem na suscetibilidade à presença de lesões oxidativas contribuindo para o desenvolvimento dessas comorbidades, dentre eles se destacam a hiperglicemia acompanhada de disfunção mitocondrial, níveis elevados de lipídeos, inflamação crônica e diminuição das defesas antioxidantes (NEGRESALVAYRE et al., 2009; AVIGNON et al., 2012; MURDOLO et al., 2013; JHA et al., 2017). Além disso, o aumento de ERO em distúrbios metabólicos como, obesidade, diabetes e SM, pode causar oxidação e mudança na conformação de enzimas importantes para neurotransmissão tais como, acetilcolinesterase (AChE), Na^+,K^+-ATPase e Ca^{2+}-ATPase, reforçando a hipótese da relação entre SM e desenvolvimento de distúrbios cognitivos e neuropsiquiátricos (ZARROS et al., 2009; STEFANELLO et al., 2013; CARVALHO et al., 2015; CARVALHO et al., 2017; SRIKANTHAN et al., 2016).

Além dos fatores anteriormente citados, tem-se observado a participação do sistema purinérgico e colinérgico no controle do processo inflamatório (UNDURUTI, 2011; SPARKS & CHATTERJEE, 2012; DE BONA et al., 2012; TOZZI & NOVAK, 2017; NOVAK & SOLINI, 2018). A adenosina trifosfato (ATP) vem sendo considerada uma molécula pró-inflamatória e pró-aterogênica, enquanto a adenosina, produto final da sua hidrólise, e a acetilcolina (ACh) desempenham ações anti-inflamatórias e neuroprotetoras (LUNKES et al., 2008; SOUZA et al., 2010; UNDURTI 2011; SPARKS & CHATTERJEE, 2012; TOZZI & NOVAK, 2017).

Vários estudos tem demonstrado que compostos bioativos produzidos pelo metabolismo secundário vegetal, tais como as antocianinas e outros compostos fenólicos apresentam ações importantes frente às complicações presentes na SM incluindo, antioxidante, anti-inflamatória, anti-hiperglicêmica, antidislipidêmica e neuroproterora (COLLINS et al. 2015; BHASWANT et al. 2015; CARVALHO et al., 2015; VENDRAME et al., 2016; ASGARY et al., 2018; JOO et al., 2018). Nesse sentido, tem sido sugerido que frutos vermelhos de *Psidium cattleianum* (araçá) e *Eugenia uniflora* (pitanga), nativos da região Sul do Brasil, são potencialmente ricos em metabólitos secundários e, consequentemente, possuem propriedades funcionais de interesse científico (JACQUES et. al., 2009; CARDOSO et al., 2018).

Tendo em vista a diversidade e a complexidade dos fatores envolvidos na patogênese da SM, a heterogeneidade molecular presente em extratos naturais os creditam como importantes agentes na terapêutica dessa síndrome. Nesse contexto, frutos vermelhos como *P. cattleianum* e *E. uniflora*, por apresentarem importantes propriedades farmacológicas e funcionais, surgem como alvos potenciais no tratamento de complicações presentes na SM.

2 Objetivos

2.1 Objetivo Geral

O objetivo geral desse trabalho foi avaliar os efeitos do consumo de extrato hidroalcoólico de frutos vermelhos nativos do Sul do Brasil sobre parâmetros metabólicos, tromboregulatórios, inflamatórios, neuroquímicos e comportamentais em ratos submetidos ao modelo de SM induzido por uma dieta hiperpalatável (DHP).

2.2 Objetivos Específicos

- Caracterizar fitoquimicamente os extratos hidroalcoólicos de frutos vermelhos de *E. uniflora* e *P. cattleianum*;
- Avaliar o efeito da administração intragástrica dos extratos de *E. uniflora* e *P. cattleianum* sobre parâmetros metabólicos (ganho de peso, gordura visceral, perfil lipídico e glicídico, níveis de ureia, ácido úrico, bem como a atividade de transaminases) em ratos submetidos ao modelo de SM;
- Avaliar o efeito dos extratos de frutos vermelhos de *E. uniflora* e *P. cattleianum* sobre parâmetros comportamentais (teste do nado forçado, do campo aberto e labirinto em cruz elevado) em ratos submetidos ao modelo de SM;
- Avaliar a ação antioxidante dos extratos de frutos vermelhos de *E. uniflora* e *P. cattleianum* através da determinação dos níveis de substâncias reativas ao ácido tiobarbitúrico, espécies reativas de oxigênio, quantificação de nitrito, conteúdo tiólico total e atividade das enzimas antioxidantes superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx) em córtex pré-frontal, hipocampo e estriado de ratos submetidos ao modelo de SM;
- Avaliar o efeito da administração dos extratos de frutos vermelhos de *E. uniflora* e *P. cattleianum* sobre a atividade das enzimas AChE, Na^+,K^+-ATPase e Ca^{2+}-ATPase em córtex pré-frontal, hipocampo e estriado de ratos submetidos ao modelo de SM;

- Avaliar o efeito dos extratos de *E. uniflora* e *P. cattleianum* sobre parâmetros inflamatórios tais como, níveis séricos de interleucina-6, atividade das enzimas NTPDase, adenosina desaminase, colinesterases em linfócitos e/ou soro de ratos submetidos ao modelo de SM;
- Avaliar o efeito dos extratos de frutos vermelhos de *E. uniflora* e *P. cattleianum* sobre parâmetros tromboregulatórios tais como, atividade das enzimas NTPDase e adenosina desaminase em plaquetas de ratos submetidos ao modelo de SM.

3. Revisão da Literatura

3.1 Síndrome Metabólica

A SM é um transtorno complexo geralmente caracterizado por um conjunto de fatores de risco para o desenvolvimento de doenças cardiovasculares e DM2 associada às condições tipicamente observadas em indivíduos com obesidade, como hiperglicemia, RI, hipertensão, obesidade visceral e dislipidemia (MAYUKO et al., 2013; DE CARVALHO VIDIGAL et al., 2013; FRANKENBERG et al., 2017; ASGARY et al., 2018). Além disso, estudos demonstram que a predisposição genética, inatividade física, tabagismo, mudanças hormonais e condições pró-inflamatórias e pró-oxidantes também podem estar relacionados com o desenvolvimento desta síndrome (SBC, 2005; DE CARVALHO VIDIGAL et al., 2013; LIN & ECKEL, 2014; SLOP et al., 2017).

Existem pelo menos dois critérios que têm sido adotados na prática clínica para a definição dos componentes presentes na SM: um proposto pelo *National Cholesterol Education Program* (NCEP), que apresentou a terceira revisão das diretrizes para diagnóstico e controle das dislipidemias, o *Adults Treatment Painel*, ATP III, conhecido como NCEP-ATP III (2001) e o outro ditado pela Organização Mundial da Saúde (OMS) (SBC, 2005). São classificados como portadores da SM pela definição do NCEP-ATP III (2001), aqueles que apresentam três ou mais dos seguintes critérios: circunferência abdominal elevada (>102 cm em homens e >88 cm em mulheres), aumento dos triglicerídeos (TG) (>150 mg/dL), diminuição do HDL (<40 mg/dL em homens e <50 mg/dL em mulheres), hipertensão arterial sistêmica (pressão arterial >130/85 mm/Hg ou uso de medicação anti-hipertensiva) e glicemia de jejum ≥ 100 mg/dL. Segundo a definição da OMS (1998), os parâmetros diagnósticos observados são: intolerância à glicose ou RI e dois ou mais dos seguintes critérios: hipertensão arterial sistêmica (pressão arterial >140/90 mm/Hg ou uso de medicação anti-hipertensiva), aumento dos TG (>150 mg/dL), diminuição do HDL (<35 mg/dL em homens e <39 mg/dL em mulheres), relação cintura/quadril elevada (> 90cm para homens e 85 cm para mulheres) e/ou índice de massa corporal (IMC) >30 Kg/m^2 e microalbuminúria (taxa de exceção de albumina >20 µg por minuto). Dentre os critérios definidos, os adotados pelo NCEP-ATP III

(2001) são os mais recomendados pela I Diretriz Brasileira de Diagnóstico e Tratamento da Síndrome Metabólica por sua simplicidade e praticidade.

Atualmente existem vários trabalhos na literatura discutindo se a SM representa apenas um conjunto de fatores de risco ou se há uma inter-relação entre eles. A falta de consenso entre os pesquisadores prediz que as alterações em cada um dos componentes pode ocasionar distúrbios em outros sistemas e assim desenvolver todo o conjunto de fatores que a compõe (DE CARVALHO VIDIGAL et al., 2013; MAYUKO et al., 2013; LIN & ECKEL, 2014; SLOP et al., 2017; ASGARY et al., 2018). Assim sendo, já está bem estabelecido que a deposição de gordura na região do abdômen causa uma série de anormalidades metabólicas que contribuem com o aumento da produção de glicose e diminuição da sensibilidade à insulina em tecidos como músculo esquelético e adiposo (SPERETTA et al., 2014; LIN & ECKEL, 2014; FRANKENBERG et al., 2017).

O aumento do tecido adiposo visceral ocasiona uma maior produção de citocinas pró-inflamatórias incluindo, TNF-α, PCR, PAI-1 e IL-6 caracterizando a SM como uma condição inflamatória crônica de baixo grau (Figura 1) (WISSE et al., 2004; BULLO et al., 2007; SAVINI et al., 2013; SPERETTA et al., 2014; LIN & ECKEL, 2014; SLOP et al., 2017; PALEY e JOHNSON, 2018). A IL-6 e o TNF-α são produzidos por muitos tipos de células em resposta a vários estímulos diferentes (WISSE et al., 2004; SLOP et al., 2017). Sabe-se que o TNF-α pode inibir a fosforilação dos receptores de insulina, resultando em defeitos na sinalização de insulina e, consequentemente, RI e desenvolvimento de DM2 (SLOP, et al., 2017). Da mesma forma, níveis elevados de IL-6 também têm sido relacionados com risco aumentado em desenvolver RI, SM, DM2 e alguns distúrbios neuropsiquiátricos (WISSE, et al., 2004; BULLO et al., 2007; SLOP et al., 2017; AMBRÓSIO, et al., 2018). Um dos efeitos mais importantes da IL-6 é o controle da produção da PCR, que por sua vez, induz a síntese de outras citocinas, como a molécula de adesão celular vascular (VCAM) e a molécula de adesão intracelular solúvel (sICAM) que podem causar injúria vascular propiciando uma ligação entre a obesidade visceral, inflamação e a aterosclerose (BULLO et al., 2007; SLOP et al., 2017).

A leptina é um hormônio proteico que está envolvido com a saciedade sendo um mediador entre a ingestão alimentar e o balanço energético

(ROMERO & ZANESCO, 2006; BERGER & POLOTSKY, 2018). Um fator importante relacionado a esse hormônio é o seu envolvimento na produção de ERO e na secreção aumentada de IL-6 e de TNF-α pelos monócitos e macrófagos (WISSE et al., 2004; BERGER & POLOTSKY, 2018). Além disso, os níveis de leptina no plasma são relacionados com o aumento do IMC e com o grau de adiposidade (ROMERO & ZANESCO, 2006; BERGER & POLOTSKY, 2018). A hiperleptinemia, encontrada em indivíduos obesos, é atribuída a alterações no receptor de leptina ou a uma deficiência em seu sistema de transporte na barreira hematoencefálica, fenômeno denominado resistência à leptina (ROMERO & ZANESCO, 2006; BERGER & POLOTSKY, 2018).

Em contraste à maioria das adipocitocinas, a expressão de adiponectina diminui com o aumento do tecido adiposo (LENZ & DIAMOND Jr., 2008; PALEY & JOHNSON, 2018). Os efeitos metabólicos desta citocina devem-se à melhora na sensibilidade à insulina, redução da gliconeogênese e aumento na captação de glicose no tecido adiposo (PALEY & JOHNSON, 2018). No músculo, estimula o metabolismo da glicose e acelera a oxidação de ácidos graxos livres (AGL) (ANTUNA-PUENTE et al., 2007; LENZ & DIAMOND Jr., 2008; BONET et al., 2009). Os efeitos anti-inflamatórios e antiaterogênicos da adiponectina devem-se à inibição do crescimento de precursores de macrófagos e sua diferenciação em células espumosas, inibição da adesão de monócitos na parede vascular por reduzir a expressão de moléculas de adesão e inibição na remodelagem e proliferação de células musculares da parede vascular (BRAY et al., 2009). Dentro deste contexto, tem sido relatado que a diminuição de tecido adiposo na região do abdômen pode afetar diretamente o balanço energético e a função da insulina por aumentar os níveis de adiponectina e diminuir os níveis de citocinas pró-inflamatórias e de ERO (BULLO et al., 2007; BUSETO et al., 2008; SAVINI et al., 2013; SLOP et al., 2017; PALEY & JOHNSON, 2018).

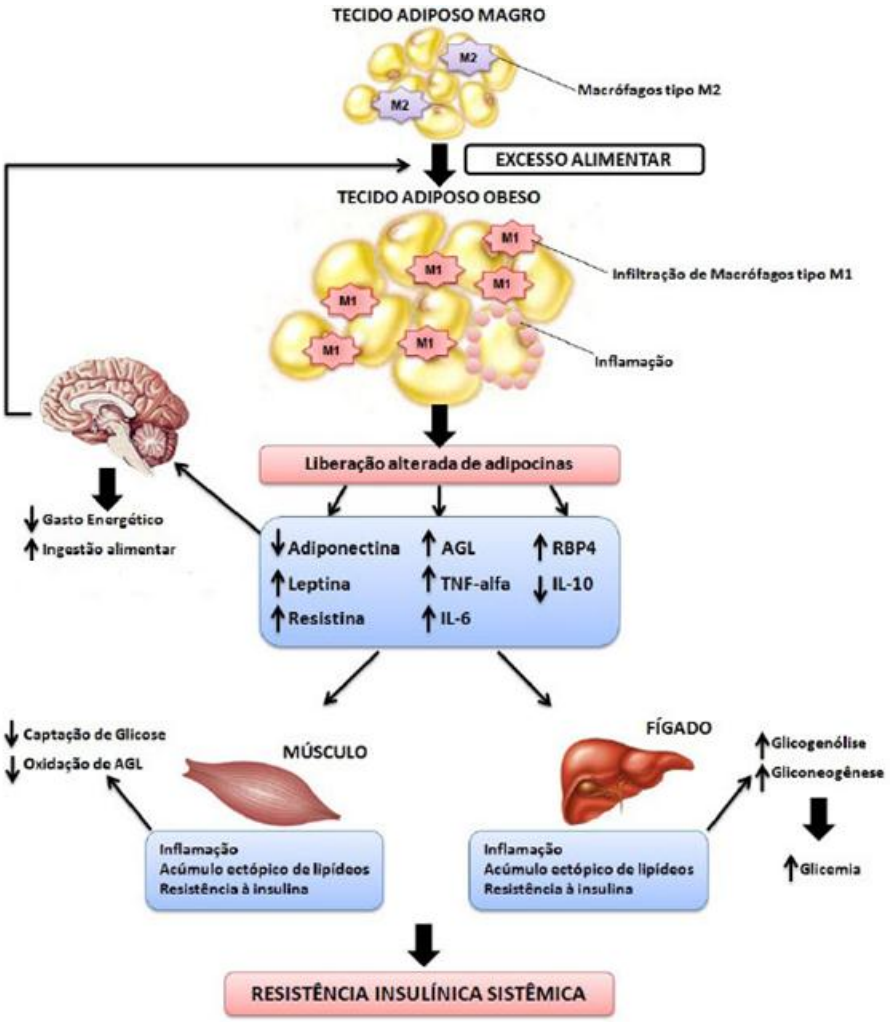

Figura 1- Mecanismos potenciais de inflamação induzida pela hipertrofia do tecido adiposo: Acúmulo de tecido adiposo visceral induz a infiltração e proliferação de macrófagos e secreção aumentada de citocinas pró-inflamatórias caracterizando a síndrome metabólica como uma condição inflamatória crônica de baixo grau. Este fato, associado ao aumento de ácidos graxos livres circulantes, leva ao aumento de ingestão alimentar, diminuição do gasto energético, além da alteração na homeostase de tecidos periféricos, como músculo e fígado contribuindo para o desenvolvimento da resistência à insulina (Adaptada de SPERETTA et al., 2014).

Devido à variedade de fatores envolvidos na SM, ela não pode ser tratada como um único agente e sim como uma combinação de fatores de risco cardiovascular (LIN & ECKEL, 2014). Nesse sentido, a melhor abordagem terapêutica para os indivíduos com SM é a mudança no estilo de vida, incluindo a redução de peso e a adoção da prática regular de exercício físico, promovendo a melhora da sensibilidade à insulina e reduzindo os riscos de complicações cardiovasculares (TUOMILETHO, 2005; LIN & ECKEL, 2014; PALEY & JOHNSON, 2018). Entretanto, é difícil iniciar e manter estilos de vida saudáveis e, em particular, com a reincidência da obesidade na maioria dos pacientes que perdem peso (LIN & ECKEL, 2014; PALEY & JOHNSON, 2018). Em seguida, agentes farmacológicos que atuam na obesidade, diabetes, hipertensão e dislipidemia podem ser usados isoladamente ou em combinação tais como, os inibidores de apetite, como derivados da fentermina e sibutramina, e inibidores da absorção de nutrientes, metformina, estatinas, fibratos, bloqueadores sistêmicos da renina-angiotensina, agonistas do peptídeo-1 semelhante ao glucagon, inibidores do transportador de glicose-2 e alguns agentes antiplaquetários (WILSON & GRUNDY, 2003; SBC, 2005; LIN & ECKEL, 2014). Porém, esses medicamentos apresentam inúmeros efeitos adversos, prejudicando a adesão ao tratamento (ECKEL et al, 2005).

Com o avanço dos estudos e conhecimento dos mecanismos envolvidos no controle da fome e saciedade, além do desenvolvimento de novas tecnologias, outros fármacos estão sendo estudados e desenvolvidos para o tratamento da SM. Dentro deste contexto, é de extrema importância a busca por alvos terapêuticos que contemplem a gama de condições presentes nessa síndrome. Estudos têm demostrado que produtos de origem natural como extratos de frutos vermelhos apresentam ações promissoras na atenuação dos fatores presentes na SM tais como, hiperglicemia, dislipidemia, RI e estresse oxidativo podendo ser um potencial agente terapêutico para o controle e prevenção dessa síndrome (CARDOSO et al., 2018; ASGARY et al., 2018).

3.2 Síndrome metabólica e sistema nervoso central (SNC)

As ERO são formadas continuamente no metabolismo celular. Essas espécies incluem não apenas radicais livres, como ânion superóxido ($O_2^{\bullet-}$), radical hidroxila ($OH^{\bullet}$) e oxigênio singleto, mas também alguns derivados não radicalares de oxigênio, como peróxido de hidrogênio (H_2O_2) e ácido hipocloroso (HClO) (HALLIWELL & GUTTERIDGE, 2007; NEGRESALVAYRE et al., 2009; RAJENDRAN et. al., 2014; HOLMSTRÖM & FINKEL, 2014). Além disso, várias fontes de ERO são identificadas, particularmente na cadeia respiratória mitocondrial, nos peroxissomos, nas enzimas oxidativas incluindo, xantina oxidase, lipoxigenases, NADPH oxidases, citocromos P450 e outras oxidases (NEGRESALVAYRE et al., 2009; HOLMSTRÖM & FINKEL, 2014). A produção dessas espécies tem sido observada em diversas condições fisiológicas, entretanto, quando formadas em excesso, podem oxidar diversas biomoléculas, como os lipídios, as proteínas e o DNA (HALLIWELL & GUTTERIDGE 2007; HOLMSTRÖM & FINKEL, 2014; RAJENDRAN et al., 2014; BETTIO et al., 2017).

A fim de evitar os efeitos deletérios das ERO, o organismo dispõe de mecanismos eficientes para a detoxificação desses agentes oxidantes, conhecidos como defesas antioxidantes (HALLIWELL e GUTTERIDGE 2007; HOLMSTRÖM & FINKEL, 2014; RAJENDRAN et al., 2014; BETTIO et al., 2017). As vias antioxidantes são sistemas de defesa que protegem o organismo de danos oxidativos, consistindo de enzimas como CAT, SOD, GPx, e numerosos antioxidantes não enzimáticos endógenos como a glutationa ou nutricionais como as vitaminas A, C e E, selênio, carotenoides e compostos fenólicos (HALLIWELL & GUTTERIDGE, 2007; HOLMSTRÖM & FINKEL, 2014; RAJENDRAN et al., 2014; BETTIO et al., 2017). Em condições fisiológicas há um balanço entre a produção de ERO e os sistemas de defesa antioxidante, no entanto, em algumas condições patológicas pode haver aumento da produção de oxidantes e/ou diminuição dos níveis de antioxidantes, resultando no que chamamos de estresse oxidativo (HALLIWELL & GUTTERIDGE, 2007; RAJENDRAN et al., 2014; BETTIO et al., 2017).

O estresse oxidativo tem sido relacionado com a fisiopatologia de várias condições relacionadas com a SM incluindo a RI, DM2, hipertensão e alguns distúrbios neuropsiquiátricos como a depressão (ROBERTS & SINDHU, 2009;

MURDOLO et al., 2013; HOLMSTRÖM & FINKEL, 2014; RAJENDRAN et. al., 2014; BONIFÁCIO et al., 2017; JHA et al., 2017). Um dos principais fatores envolvidos no aumento da produção de ERO em indivíduos com SM poderia estar relacionado com o aumento da ingestão energética (WELLEN & THOMPSON, 2010; ASCHBACHER et al., 2014; GANCHEVA et al., 2017).

De fato, a hiperglicemia crônica favorece a auto-oxidação da glicose, sendo que os açúcares oxidados podem reagir diretamente com fosfolipídeos na lipoproteína de baixa densidade (LDL), apoliproteína B (apoB) e com grupamentos amino livres de proteínas iniciando a formação de produtos finais de glicação avançada (AGEs), danificando as células e estimulando a produção de espécies reativas (NEGRESALVAYRE et al., 2009; ASCHBACHER et al., 2014; JHA et al., 2017). Ainda, a hiperglicemia favorece o aumento do fluxo da via dos polióis, o que contribui para a redução da concentração intracelular do NADPH com concomitante elevação do NADH. O NADPH é cofator da enzima glutationa redutase e, dessa forma, contribui com a elevação dos níveis de ERO por reduzir a atividade dessa enzima (HALLIWELL & GUTTERIDGE, 2007; SELVARAJU et al., 2012). Além disso, níveis elevados de glicose podem resultar em acúmulo de lipídeos. Esses, por sua vez, são capazes de induzir o estresse oxidativo por meio da geração de ERO via NADPH oxidase (NEGRESALVAYRE et al., 2009; AVIGNON et al., 2012; MURDOLO et al., 2013).

É importante destacar que as vias inflamatórias ativadas cronicamente na SM podem levar ao aumento na infiltração de macrófagos no tecido adiposo visceral e, consequentemente, secreção elevada não apenas de citocinas pró-inflamatórias, mas também de ERO (FURUKAWA et al., 2004; MURDOLO et al., 2013; BONIFÁCIO et al., 2017; GANCHEVA et al., 2017). A produção e secreção excessiva de mediadores inflamatórias e de ERO podem atravessar a barreira hematoencefálica e causar neuroinflamação (BETTIO et al., 2017; AMBRÓSIO et al., 2018) (Figura 2). Uma vez no cérebro, os mediadores inflamatórios afetam várias funções neuroquímicas envolvidas na regulação de diferentes aspectos comportamentais, incluindo consumo alimentar e saciedade, mecanismos de prazer e recompensa, níveis de energia, regulação do sono e humor (BETTIO et al., 2017; AMBRÓSIO et al., 2018). Essas disfunções são frequentemente observadas em indivíduos com obesidade e depressão e potencializam ainda mais a correlação entre essas desordens (AMBRÓSIO et

al., 2018). Além disso, é importante ressaltar que o SNC é mais suscetível ao dano oxidativo devido ao grande consumo de oxigênio, abundância de ferro, quantidades reduzidas de antioxidantes, além de conter uma grande quantidade de ácidos graxos poli-insaturados (BAIERLE et al., 2015). Nesse sentido, o dano oxidativo pode contribuir para a desmielinização e dano axonal, o que pode acarretar no comprometimento cognitivo subjacente. Esse dano é um fator crítico na patogênese de várias doenças crônicas e distúrbios neuropsiquiátricos (BAIERLE et al., 2015; MACIEL et al., 2016).

Já está bem estabelecido que o estresse oxidativo medeia processos neuropatológicos de uma série de doenças neurodegenerativas e distúrbios neuropsiquiátricos (KUMAR et al., 2012; RYBKA et al., 2013; ZHANG & YOA, 2013; BAIERLE, et al., 2015; BONIFÁCIO et al., 2017). Além disso, tem sido proposto que o aumento no consumo energético, bem como a disfunção mitocondrial também pode levar a RI no SNC (BONIFÁCIO et al., 2017; GANCHEVA et al., 2017; JHA et al., 2017). A RI no cérebro desempenha um papel importante, pelo menos como um fator que perpetua o ciclo vicioso entre a ingestão de alimentos e obesidade (GANCHEVA et al., 2017). Há também dados que fornecem evidências de que a RI está potencialmente ligada ao aumento do risco de depressão, ansiedade, doença de Alzheimer, dentre outros distúrbios neuropsquiátricos e neurodegenerativos (GANCHEVA et al., 2017; JHA et al., 2017). Ainda, a disfunção metabólica induzida pelo excesso de nutrientes pode prejudicar a neurogênese hipocampal através do aumento da peroxidação lipídica associada com disfunção oxidativa (GANCHEVA et al., 2017).

Adicionalmente, o estresse oxidativo e a inflamação crônica de baixo grau podem também alterar a atividade de enzimas como Na^+,K^+-ATPase, Ca^{2+}-ATPase e AChE que são importantes para manter a homeostase cerebral e o equilíbrio iônico (ZARROS et al., 2009; STEFANELLO et al., 2013). De fato, essas enzimas são proteínas transmembrana, conhecidas não apenas pelo bombeamento de íons, mas também por serem importantes para funções celulares e sinápticas (CARVALHO et al., 2015; CARVALHO et al., 2017; SRIKANTHAN et al., 2016). A Na^+,K^+-ATPase usa a energia da hidrólise do ATP para o transporte de Na^+ e K^+ através da membrana plasmática criando um gradiente eletroquímico que mantém o potencial de membrana (CARVALHO et al., 2015; SRIKANTHAN et al., 2016). Da mesma forma, a Ca^{2+}-ATPase

dependente da energia do ATP e sua função é regular as concentrações intracelulares de Ca^{2+}. Essa enzima contribui para as vias de sinalização desencadeadas por esse segundo mensageiro e protege as células de danos mediados por níveis elevados de Ca^{2+} intracelular (CARVALHO et al., 2015; SRIKANTHAN et al., 2016). Nesta perspectiva, estudos têm demonstrado que a cascata de sinalização de ativação dessas enzimas contribui para o agravamento da obesidade, DM2 e SM (ZARROS et al., 2009; STEFANELLO et al., 2013; SRIKANTHAN et al., 2016).

Sabe-se também que a AChE hidrolisa o neurotransmissor ACh nas sinapses colinérgicas bem como na junção neuromuscular (ZARROS et al., 2009; KADE & ROCHA 2013). Além disso, o hipocampo e regiões corticais do cérebro são os principais locais para transmissão colinérgica envolvidos na plasticidade sináptica (MACIEL et al., 2016). Adicionalmente, tem sido observado que os níveis de ACh no cérebro também podem ser afetados pelo peso corporal, hiperglicemia e estresse oxidativo (ZARROS et al., 2009; DA SILVA BENETTI et al., 2014). De fato, o aumento de ERO pode levar ao aumento na atividade da AChE e que a disfunção colinérgica pode estar relacionada às alterações cognitivas e neuroquímicas presentes na SM (ZARROS et al., 2009; STEFANELLO et al., 2013; MACIEL et al., 2016).

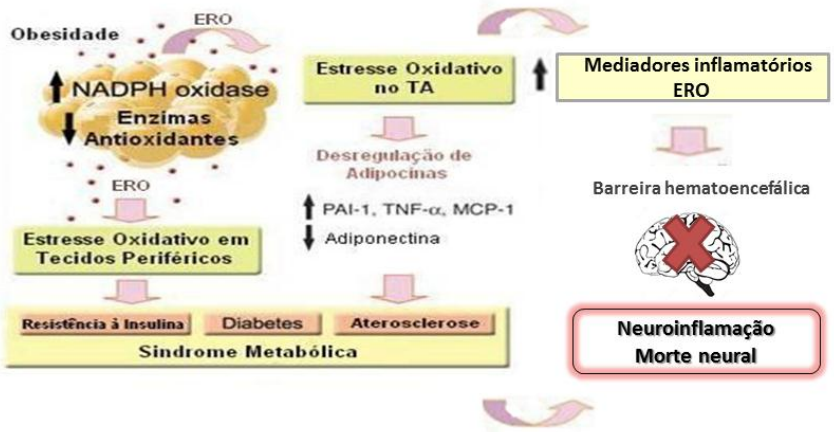

Figura 2- Esquema ilustrando como o aumento do tecido adiposo pode levar a produção aumentada de espécies reativas de oxigênio (ERO) e de mediadores inflamatórios, os quais estão relacionados com a fisiopatologia de várias condições presentes na síndrome metabólica (Adaptado de FURUKAWA et al., 2004).

3.3 Síndrome metabólica e alterações periféricas

Os mecanismos subjacentes à patogênese da SM são complexos, pois podem ser influenciados por diferentes vias biológicas incluindo inflamação e estresse oxidativo as quais podem contribuir para eventos cardiovasculares e sistêmicos (DE BONA et al., 2012; MURDOLO et al., 2013; ASCHBACHER et al., 2014; GANCHEVA et al., 2017). Durante o processo inflamatório diversos sistemas de controle estão envolvidos, incluindo o purinérgico e colinérgico (UNDURUTI, 2011; SPARKS & CHATTERJEE, 2012; DE BONA et al., 2012; DE BONA et al., 2013).

Os nucleotídeos como ATP, adenosina difosfato (ADP) e adenosina monofosfato (AMP) atuam como mensageiros extracelulares modulando a resposta imune e inflamatória (SPARKS & CHATTERJEE, 2012). Uma vez no meio extracelular, esses nucleotídeos interagem com receptores purinérgicos específicos que estão presentes na membrana plasmática de células do sistema imune (SPARKS & CHATTERJEE, 2012). Esses receptores são divididos em dois grupos: aqueles acoplados a proteína G (P2Y) e aqueles ligados a canais iônicos (P2X), sendo que já foram identificados oito tipos de receptores P2Y (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13 e P2Y14) e sete tipos de receptores P2X (P2X1-P2X7) (SPARKS & CHATTERJEE, 2012; NOVAK & SOLINI, 2018). Do ponto de vista fisiológico, a concentração extracelular de nucleotídeos é mantida baixa para minimizar a sinalização purinérgica (SPARKS & CHATTERJEE, 2012). No entanto, a hiperglicemia crônica, fator presente em indivíduos com SM, estimula diretamente a liberação de ATP para o meio extracelular (LUNKES et al., 2008; SPARKS & CHATTERJEE, 2012). O ATP é instável na circulação sendo rapidamente degradado para ADP, AMP e adenosina, por ectonucleotidases específicas (SPARKS & CHATTERJEE, 2012).

As ectonucleotidases são expressas na superfície de várias células, incluindo plaquetas e linfócitos e desempenham um papel importante na modulação purinérgica (SPARKS & CHATTERJEE, 2012; MARTINS et al., 2016b). Esse complexo enzimático inclui as enzimas E-NTPDase responsáveis pela hidrólise de ATP em ADP e AMP e a enzima 5'-nucleotidase que converte AMP em adenosina que é hidrolisada pela adenosina desaminase (ADA) em inosina (MARTINS et al., 2016b) (Figura 3). O ATP é capaz de mediar à resposta

imune atuando como um agente pró-inflamatório através da estimulação e proliferação de linfócitos e liberação de citocinas (SPARKS & CHATTERJEE, 2012; MARTINS et al., 2016a; TOZZI & NOVAK, 2017; NOVAK & SOLINI, 2018). Além disso, tem sido descrito que os receptores P2X7R são expressos no tecido adiposo e pancreático e podem desencadear a inflamação nesses tecidos (TOZZI & NOVAK, 2017; NOVAK & SOLINI, 2018). Esse fato poderia explicar o envolvimento da sinalização purinérgica no desenvolvimento da RI e na liberação de citocinas pró-inflamatórias em indivíduos com SM (MADEC et al., 2011; TOZZI & NOVAK, 2017; NOVAK & SOLINI, 2018). Além disso, em animais tem sido observada uma relação entre os receptores P2X7R e o aumento do peso corporal, hipertrofia dos adipócitos bem como o acúmulo de gordura ectópica (NOVAK & SOLINI, 2018). Adicionalmente, sugere-se que o ATP e o ADP podem induzir agregação plaquetária contribuindo, dessa forma, com o desenvolvimento de doenças cardiovasculares (BAGATINI et al., 2008; LUNKES et al., 2008; SOUZA et al., 2010; MARTINS et al., 2016b). Ainda, a hiperglicemia crônica, por promover alterações nos níveis de ATP/ ADP, pode ser um fator importante envolvido na hiperatividade das plaquetas (LUNKES et al., 2008). Além disso, a hipercolesterolemia pode levar ao acúmulo de plaquetas nas lesões ateroscleróticas, levando ao recrutamento de plaquetas adicionais para formação do trombo, indicando que a parede arterial pode assumir um fenótipo inflamatório e pró-trombogênico (DUARTE et al., 2007).

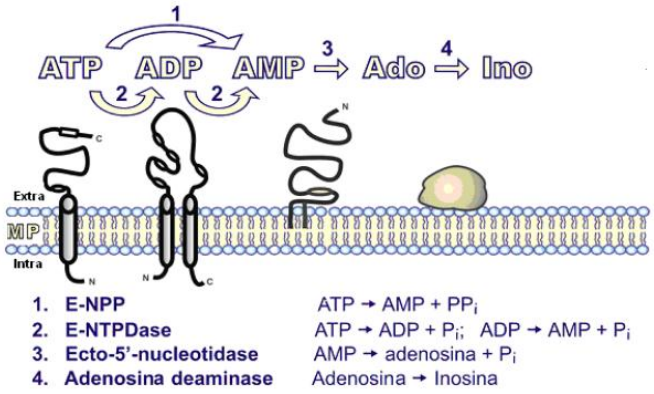

Figura 3- Cascata das ectonucleotidases responsáveis pela hidrólise de nucleotídeos de adenina e adenosina. (Adaptado de YEGUTTKIN, 2008).

Em contraste com o que ocorre com os nucleotídeos de adenina, a adenosina, seu nucleosídeo correspondente apresenta um papel modulador em várias funções fisiológicas, incluindo a inibição da agregação plaquetária, bem como ações anti-inflamatórias e imunossupressoras (SPARKS & CHATTERJEE, 2012; MARTINS et al., 2016b; TOZZI & NOVAK, 2017). Além disso, esse nucleosídeo pode regular vários fatores presentes na SM, como o metabolismo da glicose e dos lipídeos (DE BONA et al., 2012; TOZZI & NOVAK, 2017). A adenosina pode se ligar a quatro diferentes receptores acoplados à proteína G (A1, A2A, A2B e A3) e dados na literatura sugerem que os adipócitos expressam todos os tipos de receptores (TOZZI & NOVAK, 2017). Nesse sentido, os receptores A1R regulam a lipólise e, portanto, os níveis de AGL e assim podem apresentar um papel significativo na fisiopatologia da RI, diabetes e doenças cardiovasculares (DE BONA et al., 2012; TOZZI & NOVAK, 2017).

Além dos nucleotídeos, a ACh também atua na modulação da imunidade através da sua ação em receptores nicotínicos na superfície dos linfócitos e, portanto, pode apresentar efeitos anti-inflamatórios (SRIDHAR et al., 2010; UNDURTI, 2011; De BONA et al., 2012). Esse efeito pode ser atribuído à inibição da secreção de citocinas pró-inflamatórias através da estimulação do nervo vago (UNDURTI, 2011; SHENHAR-TSARFATY et al., 2013). Esse mecanismo depende do receptor α7 nicotínico (α7nAChR), que inibe a translocação do fator nuclear kappa B (NF-κB) e suprime a liberação de citocinas por monócitos e macrófagos (UNDURTI, 2011; SHENHAR-TSARFATY et al., 2013). Dessa forma, a ativação parassimpática do vago inicia como um processo anti-inflamatório denominado "reflexo colinérgico". A ativação desse reflexo pode minimizar o processo inflamatório em várias patologias dentre elas o diabetes e a SM (UNDURTI, 2011; SHENHAR-TSARFATY et al., 2013).

As concentrações de ACh são moduladas pela ação das colinesterases. A AChE hidrolisa preferencialmente ésteres com grupamento acetil e está presente nas junções neuromusculares e nas sinapses do SNC e periférico. Já a butirilcolinesterase (BuChE) está presente em vários tecidos, incluindo sangue , fígado, coração, endotélio vascular e SNC e hidrolisa outros tipos de ésteres como a butirilcolina (SHENHAR-TSARFATY et al., 2013; DA SILVA BENETTI et al., 2014; DUCHNOWICZ et al., 2018). Análises bioquímicas e neurofisiológicas demonstram um aumento na atividade simpática, diminuição na atividade do

parassimpático e diminuição na atividade vagal em pacientes com desordens metabólicas como diabetes, SM e obesidade (STRAZNICKY et al., 2008; SHENHAR-TSARFATY et al., 2013). Uma possível causa de atividade vagal prejudicada é o aumento da hidrólise da ACh mediada pelas enzimas colinérgicas observada em indivíduos com essas desordens metabólicas (STRAZNICKY et al., 2008; SRIDHAR et al. 2010; De BONA et al., 2013). Quando há um aumento na atividade das enzimas colinérgicas, ocorre degradação da ACh, a redução dessa molécula pode diminuir seu efeito anti-inflamatório (SRIDHAR et al., 2010; De BONA et al., 2012). Além disso, tem sido demonstrado que a atividade aumentada da BuChE pode estar envolvida na fisiopatologia de diferentes fatores presentes na SM, como a RI, dislipidemia e aumento da gordura visceral (SRIDHAR et al., 2010; DE BONA et al., 2013; DA SILVA BENETTI et al., 2014; DUCHNOWICZ et al., 2018).

Assim sendo, o estudo das ações desempenhadas pelos sistemas colinérgico e purinérgico na SM torna-se de extrema importância a fim de melhor compreender a fisiopatologia dessa síndrome. Ademais, a caracterização de novos marcadores inflamatórios e tromboregulatórios podem auxiliar na identificação de alternativas terapêuticas uteis na prevenção e tratamento dessa desordem metabólica.

3.4 Frutos nativos: propriedades farmacológicas

Os estudos com frutos nativos da região Sul do Brasil tiveram seu início na década de 80, nos três estados dessa região do país. No Rio Grande do Sul, as pesquisas realizadas pela Embrapa Clima Temperado inicialmente tinham o objetivo de conservação das espécies (FRANZON, 2004; FETTER et al., 2010; MEDINA et al., 2011). No entanto, com o passar dos anos cresceu a ideia de se utilizar algumas dessas espécies como complementares aos sistemas produtivos da região e, no ano de 2001, começaram os estudos de caracterização e potencialidade desses frutos (FRANZON, 2004; FETTER et al., 2010; MEDINA et al., 2011).

Nas regiões do sul do Brasil, dentre as muitas espécies frutíferas nativas existentes destacam-se aquelas da família *Myrtaceae*. Essa família compreende aproximadamente 102 gêneros, incluindo *Eugenia*, *Myrcianthes*, *Campomanesia* e *Psidium*, e 3024 espécies conhecidas, distribuídas e cultivadas, principalmente em países de clima tropical e subtropical (FRANZON et al., 2009). Entretanto, algumas espécies da família *Myrtaceae* também ocorrem em regiões de clima temperado (FRANZON et al., 2009).

A *Eugenia uniflora* L. conhecida popularmente como pitangueira é uma planta da família das mirtáceas, que tem seu cultivo disseminado na América Latina subtropical (ADEBAJO et al., 1989; MIGUES et al., 2018). Ela cresce principalmente no Brasil, razão pela qual é conhecida como “Cereja Brasileira”, mas também é cultivada na Argentina, Paraguai e Uruguai (CONSOLINI et al., 2002; MIGUES et al., 2018). Essa planta começou a ser estuda devido aos benefícios à saúde atribuída às folhas, pois essas têm sido utilizadas popularmente no tratamento de inúmeras desordens, tais como, hipercolesterolemia, gota, doenças digestivas, hepáticas e inflamatórias (SCHMADA-H, 1988; WEYERSTAHL et al., 1988; MIGUES et al., 2018).

O gênero *Psidium* é representado por aproximadamente 120 a 150 espécies, das quais algumas tem sido objeto de estudo principalmente nos componentes que dão aroma aos frutos (RASEIRA & RASEIRA, 1990; RASEIRA, 2004; FRANZON et al., 2009). O araçazeiro (*P. cattleianum* Sabine), também conhecido pelos nomes de araçá, araçá-do-mato e araçá-do-campo, apresenta-se como uma das espécies com importância econômica da família

das mirtáceas, ocorrendo em extensa área desde Minas Gerais até o Rio Grande do Sul (RASEIRA & RASEIRA, 1990; RASEIRA, et al., 2004; FRANZON et al., 2009). O fruto araçá é uma baga de coloração amarela ou vermelha, de acordo com o genótipo, cuja maturação dos frutos ocorre nos meses de fevereiro a abril na região de Sul do Rio Grande do Sul (RASEIRA & RASEIRA, 1990).

Os estudos envolvendo frutos vermelhos de *P. cattleianum* (Figura 4) e *E. uniflora* (Figura 5) tem aumentado uma vez que são potencialmente ricos em metabólitos secundários biologicamente ativos capazes de promover a saúde e retardar o aparecimento de incapacidades associadas a doenças crônicas (PROTEGGENTE et. al., 2002; SUN et. al., 2002; CARDOSO et al., 2018; MIGUES et al., 2018; ASGARY et al., 2018). Corroborando com o que foi mencionado, Medina e colaboradores (2010) demonstraram *in vitro* uma atividade antimicrobiana, antiproliferativa e antioxidante de extrato de *P. cattleianum*. Velázquez e colaboradores (2003) também relataram que extratos de *E. uniflora* apresentaram uma atividade inibitória sobre a lipoperoxidação em ratos. Ainda, extratos de frutos vermelhos de *E. uniflora* e *P. cattleianum* nativos do Sul do Brasil exibiram ação antioxidante, anti-hiperglicêmica e antidislipidêmica em animais submetidos a um modelo de RI (CARDOSO et al., 2018).

Figura 4- Frutos vermelhos de araçá (*P. cattleianum*). (FRANZON et al., 2009).

Figura 5- Frutos vermelhos de pitanga (*E. uniflora*). (FRANZON et al., 2013).

Pesquisas relacionadas aos fitoquímicos presentes nos frutos vermelhos demonstram que os compostos fenólicos desempenham ações importantes nas complicações presentes na SM incluindo redução do peso, glicose, TG, colesterol total, LDL-colesterol (COLLINS et al., 2015; BHASWANT et al., 2015; VENDRAME et al., 2016). Embora, os mecanismos relacionados às ações desses compostos não estão bem elucidados, tem sido sugerido que podem inibir enzimas digestivas, como a α-amilase e a absorção de glicose no intestino (HANHINEVA et al., 2010).

Os compostos fenólicos representam a principal classe de metabólitos secundários presentes nas plantas e estão amplamente distribuídos na natureza (PROCHÁZKOVÁ et al., 2011; GONZÁLEZ-CASTEJÓN & RODRIGUEZ-CASADO, 2011). Além disso, eles podem ser definidos como substâncias que possuem um anel aromático com um ou mais grupos hidroxilas (PROCHÁZKOVÁ et al., 2011; GONZÁLEZ-CASTEJÓN & RODRIGUEZ-CASADO, 2011). Os maiores grupos de fenólicos dietéticos são os ácidos fenólicos, os flavonoides e os taninos (PROCHÁZKOVÁ et al., 2011; GONZÁLEZ-CASTEJÓN & RODRIGUEZ-CASADO, 2011).

Os ácidos fenólicos caracterizam-se por terem um anel benzênico, um grupamento carboxílico e um ou mais grupamentos de hidroxila podendo ser encontrado na forma de ésteres, glicosídeos e amidas (SIMÕES et al., 2007). Esses compostos tem demostrado uma ampla gama de propriedades

farmacológicas como antidislipidêmicos, anticancerígenos e anti-inflamatórios (GONZÁLEZ-CASTEJÓN & RODRIGUEZ-CASADO, 2011). Já os flavonoides constituem uma grande família de fitocompostos distintos identificados em muitas frutas e vegetais, classificados em diferentes subclasses, de acordo com sua estrutura química (GONZÁLEZ-CASTEJÓN & RODRIGUEZ-CASADO, 2011; BENSALEM et al., 2018). Embora, os flavonoides não são considerados agentes nutritivos, eles também estão ganhando interesse devido ao seu papel potencial na prevenção de várias desordens incluindo, diabetes, doenças cardiovasculares e neurodegenerativas (MLADĚNKA et al., 2010; GONZÁLEZ-CASTEJÓN & RODRIGUEZ-CASADO, 2011; BENSALEM et al., 2018). Dessa forma, tem sido proposto que os flavonoides podem proteger as células de diferentes agressões que levam à morte celular, uma vez que atenuam a progressão das doenças associadas com estresse oxidativo (LAGOA et al., 2011). Assim sendo, Lagoa e colaboradores (2011), observaram que a suplementação de extrato de frutos de uva e mirtilo rico em flavonoides diminui a produção de H_2O_2 em mitocôndrias cardíacas e cerebrais dos animais tratados. Além disso, tem sido demonstrado que os flavonoides podem inibir diretamente algumas enzimas formadoras de ERO incluindo, xantina oxidase, NADPH oxidase e lipoxigenases (MLADĚNKA et al., 2010). Adicionalmente, Bensalem e colaboradores (2018) demonstraram que a suplementação de extrato rico em flavonoides melhorou o desempenho cerebral, a plasticidade e a memória em camundongos idosos podendo ser benéfico para manter funções cognitivas durante o processo de envelhecimento. Ainda, tem sido demostrado que a quercetina exibe ações anti-inflamatórias, uma vez que foi capaz de modular de forma positiva a atividade de enzimas do sistema colinérgico e purinergico em animais expostos a metais pesados (ABDALA et al., 2014).

As antocianinas pertencem à classe dos flavonoides e são responsáveis pela maioria das cores azul, violeta e quase todas as tonalidades de vermelho que aparecem em flores, frutos e algumas folhas e caules de plantas (SIMÕES et. al., 2007). Na dieta humana, são encontradas em maior abundância em frutos vermelhos e roxos e também em alguns vegetais (GONZÁLEZ-CASTEJÓN & RODRIGUEZ-CASADO, 2011). Estudos têm demonstrado que as antocianinas podem atenuar a dislipidemia por afetar favoravelmente o perfil lipídico no plasma (ASGARY et al., 2018; JOO et al., 2018). Além disso, elas podem exibir

importantes ações anti-inflamatórias e neuroprotetoras (CARVALHO et al., 2015; JOO et al., 2018). Muitos estudos demonstram que a cianidina-3-*O*-glicosídeo, antocianina mais comum em alimentos, pode apresentar ações importantes incluindo antitrombótica e anti-inflamatória além de reduzir o ganho de peso e prevenir o desenvolvimento da RI (YU et al., 2014; CASSIDY et al., 2015; YOU et al., 2017). Um dado importante a ser mencionado é que essa antocianina pode aumentar a oxidação de ácidos graxos através da sinalização da proteína quinase ativada por AMP (AMPK). A ativação da AMPK leva à fosforilação e inativação da acetil-CoA carboxilase, aumentando a oxidação dos ácidos graxos e consequentemente diminuindo a gordura visceral e melhorando o metabolismo da glicose (GUO et al., 2012; BHASWANT et al., 2015). Adicionalmente, Pei e colaboradores (2018) demostraram que a suplementação de 200 mg/kg/dia de cianidina-3-*O*-glicosídeo durante 8 semanas foi capaz de prevenir a esteatose hepática e regular a secreção de citocinas pro-inflamatórias pelo tecido adiposo em animais que consumiram uma dieta rica em gordura e colesterol.

Considerando a variedade de ações potenciais já conhecidas dos compostos fenólicos em geral, nesse estudo pretendemos verificar outros efeitos biológicos desses fitoquímicos presentes em frutos vermelhos nativos do Sul do Brasil. Essas descobertas podem contribuir com os tratamentos empregados para SM.

4. Artigo 1

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Eugenia uniflora fruit (red type) standardized extract: a potential pharmacological tool to diet-induced metabolic syndrome damage management

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Abstract

The aim of this study was to investigate the effect of *Eugenia uniflora* fruit (red type) extract on metabolic status, as well as on neurochemical and behavioral parameters in an animal model of metabolic syndrome induced by a highly palatable diet (HPD). Rats were treated for 150 days and divided into 4 experimental groups: standard chow (SC) and water orally, SC and *E. uniflora* extract (200 mg/Kg daily, p.o), HPD and water orally, HPD and extract. Our data showed that HPD caused glucose intolerance, increased visceral fat, weight gain, as well as serum glucose, triacylglycerol, total cholesterol and LDL cholesterol; however, *E. uniflora* prevented these alterations. The extract decreased lipid peroxidation and prevented the reduction of superoxide dismutase and catalase activities in the prefrontal cortex, hippocampus and striatum of animals submitted to HPD. We observed a HPD-induced reduction of thiol content in these cerebral structures. The extract prevented increased acetylcholinesterase activity in the prefrontal cortex caused by HPD and the increase in immobility time observed in the forced swimming test. Regarding chemical composition, LC/MS analysis showed the presence of nine anthocyanins as the major compounds. In conclusion, *E. uniflora* extract showed benefits against metabolic alterations caused by HPD, as well as antioxidant and antidepressant-like effects.

Keywords: metabolic syndrome; *Eugenia uniflora*; phenolic compounds; pharmacological management

1. Introduction

Metabolic syndrome (MetS) is a major health problem, which predisposes to the development of type 2 diabetes, cardiovascular and kidney diseases [1]. It is characterized by the presence of three or more of the following risk factors: hypertension, hyperglycemia, dyslipidemia, obesity and insulin resistance (IR) [1]. The prevalence of MetS is rapidly increasing worldwide including in developing countries, which is due primarily to prevailing sedentary lifestyles and unhealthy eating habits [2].

Experimental studies in animal models have shown that chronic consumption of diets with a high carbohydrate and fats content mimics the main signs of MetS in humans, especially dyslipidemia, hyperglycemia, glucose intolerance, obesity and IR [3]. Moreover, the consumption of these diets may increase fat deposits and free fatty acid (FFA) levels in the tissues and circulation leading to the development of a pro-inflammatory and pro-oxidant state [4].

It is known that the increase of reactive oxygen species (ROS) as well as pro-inflammatory cytokines is directly related to the development of MetS [5]. Moreover acetylcholinesterase (AChE) may contribute to the pathways controlling inflammatory and immune responses [6] and some reports demonstrated that cholinesterase activities were altered in diabetes and MetS [7]. In addition, there is a growing appreciation that the complications of MetS and obesity may result in increased risk for neurological co-morbidities like depressive illness [8]. In this context, studies indicate that oxidative stress mediates the neuropathological processes in a series of neurodegenerative diseases, and neuropsychiatric disorders [9].

Considering the variety of factors involved in the development of MetS the search for potential new treatments still remains relevant. In this way, epidemiological studies have suggested an inverse relation between the consumption of polyphenol-rich foods and the risk of degenerative diseases. Therefore, there has been a great deal of interest in the screening and characterization of novel potentially therapeutic compounds of polyphenol-rich extracts obtained from foods and medicinal plants [10].

Eugenia uniflora (*Myrtaceae)* is widely distributed throughout South America [11]. Studies with its leaf extracts have shown numerous actions such as antioxidant and anti-inflammatory effects, reduction of triacylglycerol (TAG), reduced weight gain and decreased glucose levels in the blood [11,12]. However, the lack of studies related to the pharmacological properties of *E. uniflora* fruits, despite the presence of anthocyanins, polyphenols as major chemical constituents, make this fruit a source of antioxidant compounds, which are desirable to the patients with MetS [13]. In this sense, this work aimed at evaluating the effects of *E. uniflora* fruit (red type) extracts on metabolic status, as well as on neurochemical and behavioral parameters in rats fed with a highly palatable diet.

2. Materials and Methods

2.1. Phytochemicals

2.1.1. Extraction

E. uniflora fruits (red type) were harvested in an orchard belonging to *Embrapa Clima Temperado* (Brazilian Agricultural Research Corporation) Pelotas/RS, Brazil (31º40’47”S and 52º26’24”W). After picking, the fruits were

immediately frozen at -20ºC and protected from light. The extracts were prepared according to Bordignon et al., [14] with modifications. Briefly, unprocessed frozen *E. uniflora* fruits (30 g) were sonicated for 30 min at 25ºC in 90 mL 70:30 v/v ethanol-water (pH 1.0). The crude extracts were filtered; the ethanol removed under reduced pressure and then lyophilized.

2.1.2. Total phenolic, flavonoid and anthocyanin contents

The total phenolic content was determined according to Singleton et al., [15] with minor modifications and expressed as milligrams of gallic acid per 1 g of dried extract. The total flavonoid content was determined as described by Miliauskas et al., [16] and results were expressed as milligrams of gallic acid per 1 g of dried extract. Anthocyanins were quantified by the pH differential method [17] and reported as milligrams of cyanidin-3-glucoside per 1 g of dried extract. Data are mean ± SD and all analyses were performed in triplicate.

2.1.3. Identification of anthocyanins by LC/PDA/MS/MS

Anthocyanins were identified using an Acquity-UPLC^{TM} coupled to a photodiode array detector (PDA) and to a high-resolution mass spectrometer (Xevo$^{®}$ G2 QTof model – WATERS$^{®}$). The chromatographic separation was performed using a C18 chromatography column (Synergi^{TM}- Phenomenex®) 4 µm, 150 x 2.0 mm at 40^{o}C, and the injection volume was 5 µL. The elution was carried out using an aqueous solution of formic acid 2% (solvent A) and acetonitrile with 1% of formic acid (solvent B). The method used a linear gradient at constant flow (0.4 mL.min^{-1}). The total time of analysis was 34 min according to the following conditions: 0-10 min, 5-12% B; 10-29 min, 12-18% B; 29-33 min,

18% B; 33-34 min, 5% B. Detection was performed at 520 nm, and the range of spectral scanning in the visible region was 450 – 600 nm (PDA). Mass spectrometry detection was carried out with an electrospray ionization source (ESI) set on negative ion mode, capillary voltage, 1.0 kV, source block temperature, 120°C, desolvation temperature, 600°C; nebulizer nitrogen flow rate, 80 L h^{-1}, desolvation nitrogen gas flow, 800 L h^{-1}, and cone voltage, 40 V, controlled by MassLynx v.4.1 software for data acquisition and processing. The mass scanning ranged from *m/z* 200 to 1500 with a scan time of 0.5 s. MS/MS analysis was performed using a collision energy ramp (10 – 30 eV);and with argon as collision gas. All analyses were performed in triplicate.

2.2 Animals and drug treatments

Male Wistar rats aged 21 days maintained at 21-25°C with free access to water and food, under a 12:12 h light:dark cycle were used throughout this study.

Rats were divided into four groups: (1) standard chow group (SC) + vehicle, which received standard laboratory rat chow (50% carbohydrate, from starch, 22% protein and 4% fat) and water orally; (2) SC + *E. uniflora*, which received standard chow and 200 mg/Kg/day of *E. uniflora* orally; (3) highly palatable diet group (HPD) + vehicle, which received an enriched sucrose diet (65% carbohydrates 34% being from condensed milk, 8% from sucrose and 23% from starch, 25% protein and 10% of fat) and water orally (4) HPD + *E. uniflora*, 200 mg/Kg/day orally. The HPD and dose of *E. uniflora* extract used in this study were chosen according to Oliveira et al., [18]. The experiments were performed after the approval of the local Ethics Committee (CEEA n° 9125) and no efforts were spared to minimize animal suffering.

2.3 Body weight gain and food intake

Changes in body weight and food intake patterns of rats were measured throughout the experimental period. The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study. The quantity of food consumed by each group was recorded weekly, and the food consumption per rat was calculated for all groups.

2.4 Sample collection and biochemical assay

After 150 days of food and extract administration and 24 h after the last behavioral test, the animals were euthanized by decapitation having fasted for 6 h. At euthanasia, visceral fat was weighed and the blood collected. Serum was obtained by centrifugation at 4000 rpm (4°C) for 15 min. Liver and adipose tissue were collected for further histopathological analysis. Prefrontal cortex, hippocampus and striatum were collected and stored at -80°C for subsequent biochemical analyses.

2.4.1 Glucose tolerance test

Rats were injected intraperitoneally with a 50% glucose solution load of 2 mg/g of body weight. The glucose levels for all the groups were estimated by the glucometer (AccuChek Active, Roche Diagnostics®, USA) at 30, 60, and 120 min after the injection by a small tail puncture.

2.4.2 Serum biochemical parameters

Measurements of serum glucose, total cholesterol, cholesterol-LDL, cholesterol-HDL, TAG, urea, uric acid and alanine aminotransferase (ALT) levels were determined using commercially available diagnostic kits supplied by Labtest® (Labtest, MG, Brazil).

2.4.3 Histopathological analysis

Liver and adipose tissue samples were fixed in 10% buffered formalin (pH 7.4) and embedded in paraffin, cleaved and routinely processed. Samples were cut into 5-µm sections and stained with hematoxylin and eosin. Histological analyses were performed in all groups.

2.5 Behavioral analysis

2.5.1 Forced Swim Test (FST)

The depressive-like behavior was evaluated by the total duration of immobility in the forced swimming test, as previously described by Kaster et al., [19].Rats were individually forced to swim in an open cylindrical container, with water at 25 ± 1°C and the total duration of immobility during a 5 min period was scored: rats were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their head above water.

2.5.2 Open-field Test

The ambulatory behavior was assessed in an open-field test as previously described by Kaster et al., [20]. The apparatus consisted of a box with the floor of the arena divided into 12 equal squares and placed in a sound-free room.

Animals were placed in the rear left square and left to freely explore it for 5 min during which time the number of squares crossed with all paws (crossing) was counted.

2.5.3 Elevated plus-maze

Anxiety-related behaviors were evaluated using the elevated-plus maze (EPM). Animals were placed in the center of the EPM and were free to explore for 5 min. The number of entries into the open and closed arms, as well as time spent in the arms was measured during the 5-minute test. To evaluate the animal's anxiety we used an index calculated with the following equation. Anxiety index values range from 0 to 1, with a higher value indicating increased anxiety [21].

Eq. 1: Anxiety index = 1 – [(open arm time/5 min) + (open arm entry/total entry)]/2

2.6 Tissue preparation

Prefrontal cortex, hippocampus and striatum were homogenized in sodium phosphate buffer pH 7.4 containing KCl. The homogenates were centrifuged at 3500 rpm for 10 min at 4ºC and the supernatant was separated and used for neurochemical analyzes. Protein was determined by the method of Lowry, et al., [22] or Bradford [23].

2.7 Oxidative stress parameters

2.7.1 Thiobarbituric acid-reactive substances (TBARS)

TBARS levels were determined according to the method described by Ohkawa et al., [24] and reported as nmol TBARS/mg protein.

2.7.2 Total thiol content assay

This assay was performed as described by Aksenov and Markesbery [25]. Results were reported as nmol TNB/mg of protein.

2.7.3 Antioxidant enzyme activities

Catalase (CAT) activity was assayed according to Aebi [26] based on the decomposition of H_2O_2. Superoxide dismutase (SOD) activity was measured by the method described by Misra and Fridovich [27] that is based on the inhibition of superoxide dependent adrenaline auto-oxidation. Glutathione peroxidase (GPX) activity was measured using commercially available diagnostic kits supplied by RANDOX (Brazil). The enzyme activities were reported as units/mg protein.

2.8 Acetylcholinesterase(AChE) activity

AChE activity was determined as described by the colorimetric method of Ellman, et al., [28] with modification and the enzyme activity was expressed as µmol/h/mg of protein.

2.9 Statistical analysis

The values were expressed as mean ± S.E.M. Glucose tolerance was analyzed by Repeated Measures analyses of variance (ANOVA) and Bonferroni's

post-hoc test. Parametric variables were tested by Two-way ANOVA and Bonferroni *post-hoc* test. A value of $P \leq 0.05$ was considered to be significant. Analyses were performed using the GraphPad PRISM 5® software.

3. Results

3.1 Phytochemical characterization

The total phenolic content was 7.92 ± 0.23 mg/g of dried extract while the total flavonoid content, 5.50 ± 0.68 mg/g of dried extract was detected. Total monomeric anthocyanin content was1.72 ± 0.05 mg/g of dried extract.

Analysis by LC/PDA/MS/MS showed the presence of nine anthocyanins (Table 1) of which just delphinidin-*O*-glucoside [29] and cyanidin-3-*O*-glucoside [29,30] were previously described for *E. uniflora* fruits. Considering the intensity of the peaks, it was possible to describe cyanidin-3-*O*-glucoside as the major anthocyanin present in these fruits. Furthermore, as far as we are aware, this is the first report of cyanidin-*O*-galactoside, petunidin-*O*-hexoside, pelargonidin-3-*O*-glucoside, pelargonidin-*O*-rutinoside, malvidin-3-*O*-glucoside, malvidin-*O*-pentoside, and malvidin-*O*-acetylhexoside in *E. uniflora* fruits.

3.2 Metabolic status

As shown in Figure 1, HPD induced an impaired glucose tolerance ($P<0.01$) and treatment with *E. uniflora* was able to prevent this change in the animals submitted to the HPD.

Table 2 shows that *E. uniflora* treatment prevented the increase of visceral fat mass (treatment: [$F(1,27) = 7.40$; $P<0.05$], HPD: [$F(1,27) = 32.34$, $P<0.001$], interaction: [$F(1,27) = 4.41$; $P<0.05$]), blood glucose levels (*E. uniflora*: [$F(1,18) =$

8.95; *P*<0.05], HPD: [F(1,18) = 14.38; *P*<0.001], interaction: [F(1,18) = 24.63*; P*<0.001]), total cholesterol (treatment: [F(1,22) = 28.43; *P*=0.001], HPD: [F(1,22) = 36.94; *P*<0.001], interaction: [F(1,22) = 24.35*; P*<0.001]), cholesterol-LDL (treatment: [F(1,14) = 44.10; *P*<0.001], HPD: [F(1,14) = 41.52; *P*<0.001], interaction: [F(1,14) = 28.11*; P*<0.001]), TAG (treatment: [F(1,13) = 16.72; *P*<0.001], HPD [F(1,13) = 6.85; *P*<0.05], interaction: [F(1,13) = 6.11*; P*<0.05]) caused by HPD. Although we did not find an interaction between groups, *E. uniflora* consumption was able to prevent the increase the weight caused by HPD (treatment: [F(1,25) = 6.60; *P*<0.05], HPD [F(1,25) = 23.44; *P*<0.001], interaction: [F(1,25) = 3.48*; P*<0.05]). However, there was no significant difference in any of the groups tested as to cholesterol-HDL, urea, uric acid and ALT (*P*>0.05).

Regarding histopathological analyses, we did not observe degenerative, necrotic or inflammatory processes in liver and adipose tissue in any of the tested groups (data not shown).

3.3 Behavioral parameters

The rats submitted to HPD protocol exhibited a significant increase in immobility time in the FST when compared to control animals, suggesting a depressive-like state. Still, the treatment with *E. uniflora* extract prevented this increase caused by HPD, suggesting an antidepressant-like effect (Fig. 2B) (treatment: [*F*(1,24) = 3.77, *P*>0.05], HPD: [*F*(1,24) = 9.63, *P*<0.05], interaction: [*F*(1,24) = 8.52, *P*<0.05]).

In order to rule out nonspecific motor effects that could influence activity in the FST, rats were also submitted to the open-field test (Fig. 2A) and no

significant change in the ambulatory behavior was observed in the open-field test (treatment: [$F(1,23) = 0.76$, $P>0.05$], HPD: [$F(1,23) = 1.11$, $P>0.05$], interaction: [$F(1,23) = 0.64$, $P>0.05$]).

We also observed that there was no difference in the anxiogenic profile of animals in any of the groups tested (anxiety index) (treatment: [$F(1,24) = 2.51$, $P>0.05$], HPD: [$F(1,24) = 0.43$, $P>0.05$], interaction: [$F(1,24)=0.67$, $P>0.05$]) (data not shown).

3.4 Neurochemical parameters

Table 3 shows that although there is no interaction between groups, *E. uniflora* treatment was able to prevent the increase of TBARS levels in hippocampus of the animals submitted to HPD. In addition, *E. uniflora per se* reduced TBARS levels when compared to control group (treatment: [$F(1,24) = 18.03$, $P<0.001$], HPD: [$F(1,24) = 57.63$, $P<0.001$], interaction: [$F(1,24) = 0.0018$, $P>0.05$]). When thiol content was evaluated no interaction between treatment and diet was observed, but it was possible to observe a significant decrease in this parameter by HPD (treatment: [$F(1,21) = 0.29$ $P>0.05$], HPD: [$F(1,21) = 14.49$, $P<0.001$], interaction: [$F(1,21) = 1.27$, $P>0.05$]). As regard the activity of antioxidant enzymes, it was observed that the *E. uniflora* treatment prevented the reduction of SOD (treatment: [$F(1,22) = 0.33$, $P>0.05$], HPD: [$F(1,22) = 11.47$, $P<0.01$], interaction: [$F(1,22) = 16.28$, $P<0.001$]) and CAT caused by HPD consumption in the hippocampus (treatment: [$F(1,18) = 0.275$, $P>0.05$], HPD: [$F(1,18) = 0.58$, $P>0.05$], interaction: [$F(1,18) = 23.52$, $P<0.001$]). GPX was not altered in any groups tested ($P>0.05$).

Additionally, we evaluated the effects of *E. uniflora* extract on oxidative stress parameters in the prefrontal cortex (Table 3). The treatment with this extract also was able to prevent the increase in TBARS levels induced by HPD, as well as in the SC group (treatment: [$F(1,18) = 5.06$, $P<0.05$], HPD: [$F(1,18) = 17.38$, $P<0.001$], interaction: [$F(1,18) = 10.86$, $P<0.05$]). Also, it was possible to observe a significant decrease in total thiol content induced by HPD (treatment: [$F(1,21) = 0.45$, $P>0.05$], HPD: [$F(1,21) = 4.63$, $P<0.05$], interaction: [$F(1,21) = 5.63$, $P<0.05$]).

In addition, *E. uniflora* treatment prevented the decreased activity of antioxidant enzymes such as SOD caused by consumption of HPD (treatment:[$F(1,17)=1.10$, $P>0.05$], HPD: [$F(1,17) = 18.70$, $P<0.001$], interaction: [$F(1,17) = 8.23$, $P<0.05$]) and CAT (treatment: [$F(1,14) = 1.39$, $P>0.05$], HPD: [$F(1,14) = 18.30$, $P<0.001$], interaction: [$F(1,14) = 7.79$, $P<0.05$]). The antioxidant enzyme GPX showed no difference in the HPD or in the group treated with *E. uniflora* extract ($P>0.05$).

Table 3 also shows the effects of *E. uniflora* extract on oxidative stress parameters in the striatum. It was observed that the extract was able to prevent the increase in TBARS levels induced by HPD, as well as in the SC group (treatment: [$F(1,28) = 19.19$, $P<0.001$], HPD: [$F(1,28) = 6.83$, $P<0.05$], interaction: [$F(1,28) = 6.16$, $P<0.05$]). Besides, the thiol content was decreased by HPD (treatment: [$F(1,18) = 0.0011$, $P>0.05$], HPD: [$F(1,18) = 6.86$, $P<0.05$], interaction: [$F(1,18) = 11.82$, $P<0.05$]) (Table 3). As regards antioxidant enzymes, *E. uniflora* treatment prevented the decreased activity of SOD caused by consumption of HPD (treatment: [$F(1,19) = 4.68$, $P<0.05$], HPD: [$F(1,19) = 5.95$, $P<0.05$], interaction: [$F(1,19) = 8.19$, $P<0.05$]) and CAT (treatment: [$F(1,25) =$

2.60, $P>0.05$], HPD: [$F(1,25) = 6.63$, $P<0.05$], interaction: [$F(1,25) = 6.33$, $P<0.05$]). No significant difference in GPX activity was observed in any of the groups tested ($P>0.05$).

As shown in Table 4, *E. uniflora* treatment prevented the increase in cortical AChE activity caused by consumption of HPD (treatment: [$F(1,19) = 20.24$, $P<0.001$], HPD: [$F(1,19) = 4.77$, $P<0.05$], interaction: [$F(1,19) = 5.07$, $P<0.05$]). In contrast, this enzyme activity was not altered in the hippocampus and striatum ($P>0.05$).

4. Discussion

MetS is commonly associated with the development of IR and hypertension, increased glucose levels, TAG and LDL-cholesterol. Although the exact mechanisms that trigger this syndrome have not been well defined, visceral obesity and IR are the main factors involved [31]. On the other hand, dietary polyphenols constitute a large family of bioactive substances, which could be an effective nutritional strategy to improve the health of patients with MetS [32].

In the present study, we investigated the ability of *E. uniflora* fruit extract to prevent some metabolic, neurochemical and behavioral parameters. Our data showed that HPD-induced MetS caused glucose intolerance, increased weight and visceral fat, glucose, total cholesterol, LDL-cholesterol and TAG levels, and also induced behavioral and neurochemical alterations. Besides, we demonstrated that the changes caused by the consumption of HPD were prevented by *E. uniflora* extract. In addition, no histopathological changes were observed in liver and adipose tissue. The diet and treatment used did not alter the metabolism of hepatocytes or adipose cells, since they did not trigger any

inflammatory or degenerative responses. In contrast, studies with high-fructose or high-fat diets demonstrated an increase in the size of adipocytes, as well as hepatic fat accumulation, fibrosis and inflammation in the liver [33, 34, 35].

Data in the literature suggest that *E. uniflora* fruits are a major source of phytochemicals, such as phenolic compounds, flavonoids and anthocyanins [36]. These compounds are known to present antioxidant, anti-inflammatory and neuroprotective properties, besides having beneficial effects on the metabolism of glucose and lipids [37]. It has been reported that anthocyanins, flavonoids and other phenolic compounds present important therapeutic effects in preventing obesity, type 2 diabetes, IR and dyslipidemia [38]. Although the mechanisms involved in these effects have not yet been elucidated, the main hypothesis of action of these chemical components of *E. uniflora* fruit extract in the prevention of IR and hyperglycemia may be attributed to the inhibition of carbohydrate digestion and absorption of glucose in the intestine, stimulation of insulin secretion by pancreatic beta cells, modulation of liver glucose secretion, activation of insulin receptors, glucose uptake in insulin sensitive tissues and modulating hepatic glucose production [37]. Manzano and Williamson [39] observed that fruit extracts containing phenolic acids, quercetin and tannin inhibited glucose transport *in vitro* via glucose active transporter-dependent sodium (SGLT1) and GLUT2 in membrane vesicles of the intestine. Hsu and Yen [40] demonstrated that supplementation of gallic acid reduced TAG, total cholesterol, LDL-cholesterol levels and visceral fat in mice fed a high fat diet. Regarding anthocyanins, it was reported that these compounds have very powerful health-promoting effects and can be used as a functional food factor [41].

It is known that the polyphenols can play an important role in neutralizing ROS, acting to prevent pathologies related to oxidative stress such as MetS [12]. In this study, we demonstrated that the consumption of HPD increased TBARS levels and decreased the total thiol content in hippocampus, prefrontal cortex and striatum. However, *E. uniflora* treatment was able to prevent the increase in TBARS levels caused by HPD in all tissues tested. Corroborating our findings, Trevino and collaborators [42] also observed an increase in hippocampal lipid peroxidation in animals subjected to a MetS model induced by high calorie diet consumption. TBARS is a marker of lipid peroxidation formed by the reaction with cytotoxic products such as malondialdehyde released during the oxidation of unsaturated fatty acids and plays an important role in the complications of MetS [43]. Lipid peroxidation induced by the consumption of HPD may be associated with increased glucose levels, which can lead to increased production of ROS by glucose autoxidation, because chronic hyperglycemia leads to the formation of end product of advanced glycation damaging cells and stimulating the production of ROS [44]. Moreover, chronic hyperglycemia present in MetS can also contribute to neuronal damage and changes in thiol groups in the brain. In this study we observed that HPD decreased total thiol content in the brain structures tested, suggesting that this animal model induces protein damage to sulfhydryl groups. Cuhad and collaborators [45] also showed a decrease in total thiol content in cerebral cortex and hippocampus of diabetic rats with cognitive deficits.

Some studies have shown an association between decreased antioxidant defenses in brain structures and increased oxidative stress in MetS and type 2 diabetes [18,44]. Therefore, we evaluated the activity of antioxidant enzymes in

the prefrontal cortex, striatum and hippocampus of animals submitted to HPD and treated with *E. uniflora* extract. Our data showed that the consumption of HPD significantly decreases the SOD and CAT activity in the brain structures tested; moreover, it was found that treatment with *E. uniflora* extract was able to prevent the reduction of the activity of these enzymes in these tissues. However, there was no significant difference in the activity of GPX enzyme in animal tissues from any of the groups tested.

It is well known that the CNS uses a significant amount of oxygen and ATP, and shows decreased antioxidant defenses compared with other tissues, resulting in a great susceptibility to oxidative stress [44]. It has also been shown that the high levels of ROS in MetS play an important role in the pathophysiology of depression [46]. In this study, HPD increased the immobility time in the FST indicating depressive-like behavior. However, treatment with *E. uniflora* extract prevented this alteration, suggesting an antidepressant-like effect. According to our findings, studies using animal models of depression have showed an increase in lipid peroxidation and decrease of antioxidant enzymes in the prefrontal cortex, striatum and hippocampus, suggesting that oxidative stress may play an important role in the relationship between MetS and depression since these structures are related to mood disorders, especially depression [18,47]. This hypothesis can be related to the antidepressant-like effect of the treatment with *E. uniflora* extract since it showed significant antioxidant action.

There is a large amount of data in the literature demonstrating that changes in the activity of AChE respond to various insults including oxidative stress and are related to the pathogenesis of a variety of diseases such as diabetes, inflammation, neurological disorders [7]. Therefore, we demonstrated

that the HPD increased AChE activity in the prefrontal cortex; however, treatment with *E. uniflora* extract was able to prevent the increased activity of this enzyme in this tissue demonstrating a neuroprotective effect. Corroborating these findings, Zarros and collaborators [7] demonstrated a significant increase in rat brain AChE activity after the induction of diabetes. Based on this, we suggest that the cholinergic dysfunction present in diabetes and MetS may be related to cognitive and neurochemical dysfunctions, however the role of AChE in depression is still little explored and controversial [7]. Furthermore, phytochemicals present in *E. uniflora* extract could contribute to a modulation of the levels of ACh in the synaptic cleft and consequent regulation of cholinergic activity involved in therapeutic strategies.

5. Conclusion

Our results demonstrate that the treatment with *E. uniflora* extract prevents alterations in metabolic parameters, oxidative damage as well as cholinergic and behavioral changes observed in MetS. Hence, this extract had an antihyperglycemic, antihyperlipidemic and a neuroprotective role since it presented antioxidant and antidepressant-like effects; further studies should be performed on the role of *E.uniflora* fruit extract in the prevention of MetS. We are currently pursuing this goal.

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Conflict of interest

The authors declare that there is no conflict of interest in the study.

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Figure captions

Fig. 1 Glucose tolerance test measured at the baseline (0), 30, 60 and 120 min after glucose injection (2 mg/g body weight). Data are expressed as mean ± S.E.M. (***) $P < 0.001$ compared to the vehicle/SC. (**) $P < 0.01$ compared to the vehicle/SC. Repeated measures ANOVA followed by Bonferroni *post-hoc* test. SC = Standard Chow; HPD = Highly Palatable Diet.

Fig. 2 Effect of *E. uniflora* extract treatment in rats exposed to a highly palatable diet on ambulatory behavior in the open-field test (A) and immobility time in the FST (B). The results are expressed as mean ± S.E.M. (n=9-10 for group). **$P<0.01$ when compared to the vehicle/SC. $^{\#}P<0.05$ when compared to vehicle/HPD. Two-way ANOVA followed by Bonferroni *post-hoc* test. SC = Standard Chow; HPD= Highly Palatable Diet.

Fig. 1

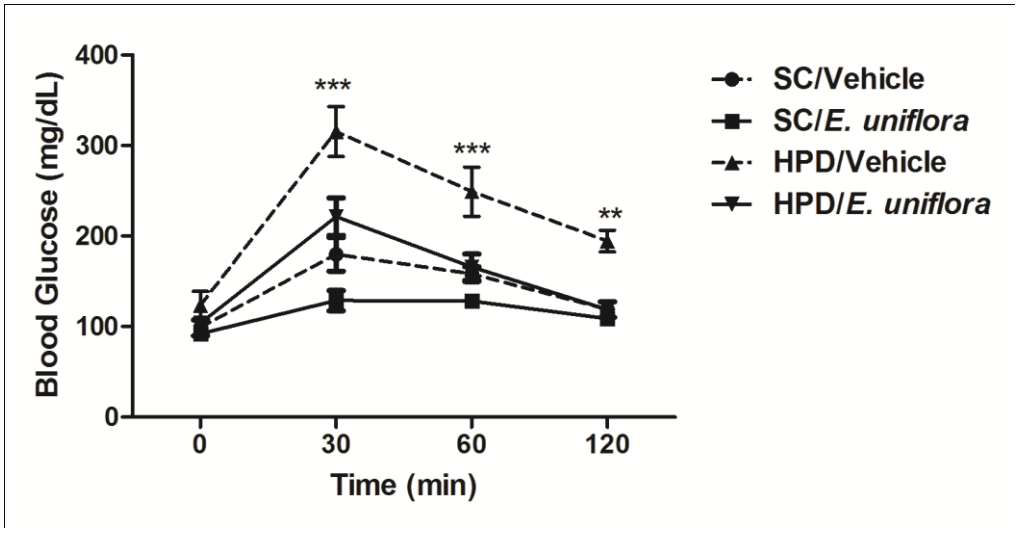
Blood Glucose (mg/dL)
400
300
200
100
0
***
***
**
0
30
60
120
Time (min)
SC/Vehicle
SC/E. uniflora
HPD/Vehicle
HPD/E. uniflora

Fig. 2

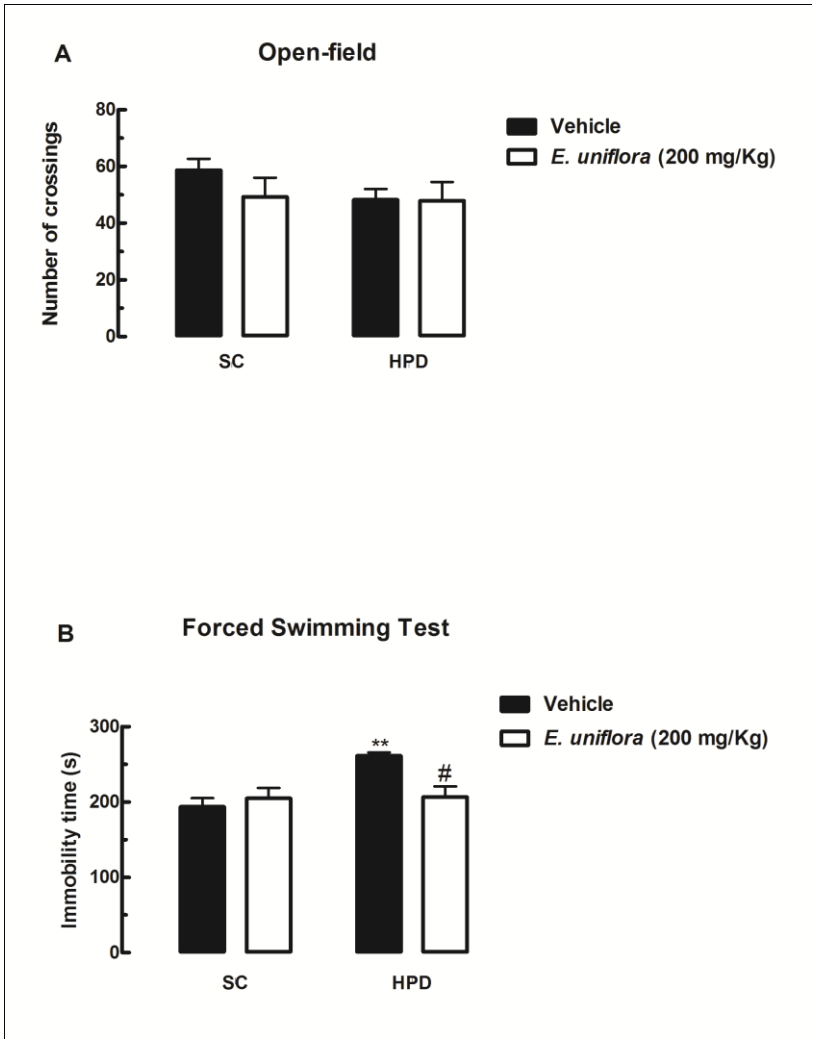
A
Open-field
Number of crossings
80
60
40
20
0
SC
HPD
Vehicle
E. uniflora (200 mg/Kg)
B
Forced Swimming Test
Immobility time (s)
300
200
100
0
**
#
SC
HPD
Vehicle
E. uniflora (200 mg/Kg)

Table 1. Chromatographic and spectroscopic profile of anthocyanins from fruits of *E. uniflora* (red type).

RT (min)	λ_{max} (nm)	$[M+H]^+$ (*m/z*)	Error (ppm)	MS/MS (*m/z*)	Identification[a]
6.50	523	465,1004	-6,2	303	Delphinidin-*O*-glucoside
7.24	515	449,1073	-2,4	287	Cyanidin-3-*O*-glucoside[a]
8.27	515	449,1065	-4,2	287	Cyanidin-*O*-galactoside
8.68	520	479,1166	-5,0	317	Petunidin-*O*-hexoside
10.07	505	433,1130	1,2	271	Pelargonidin-3-*O*-glucoside[a]
10.87	510	579,1751	6,4	271	Pelargonidin-*O*-rutinoside
11.58	523	493,1331	-3,0	331	Malvidin-3-*O*-glucoside[a]
13.84	523	463,1221	-4,1	331	Malvidin-*O*-pentoside
20.17	525	535,1471	3,6	331	Malvidin-*O*-acetylhexoside

[a]Identified by injection of corresponding authentic standards, literature data and mass spectra database (Reaxys®).

Table 2. Effect of *E. uniflora* extract treatment in rats exposed to a highly palatable diet on metabolic parameters.

	SC/Vehicle	SC/*E. uniflora*	HPD/Vehicle	HPD/*E. uniflora*
Weight gain (g)	373.20±21.35	362.57±10.64	474.62±19.64***	407.56±7.47$^{\#\#}$
Visceral fat mass (g)	15.66±1.86	13.85±0.93$^{\#\#\#}$	38.33±4.92***	24.30±1.75$^{\#\#}$
Glycemia (mg/dL)	77.40±3.79	88.98±3.48$^{\#\#\#}$	128.86±6.79***	82.10±2.84$^{\#\#\#}$
Total cholesterol (mg/dL)	105.1±3.86	104.1±4.34$^{\#\#\#}$	156.8±5.72***	109.4±1.63$^{\#\#\#}$
Cholesterol LDL (mg/dL)	46.77±2.53	40.67±7.77$^{\#\#\#}$	100.3±2.83***	45.87±4.07$^{\#\#\#}$
Cholesterol HDL (mg/dL)	49.47±1.95	55.00±3.55	43.37±3.48	51.05±2.52
Triacylglycerol (mg/dL)	65.89±10.20	55.68±6.86$^{\#\#}$	98.00±3.20*	56.60±3.66$^{\#\#}$
Urea (mg/dL)	69.99±3.88	55.98±4.62	56.02±2.48	54.29±2.32
Uric acid (mg/dL)	1.01±0.09	1.02±0.04	0.75 ±0.12	0.84±0.21
ALT (mg/dL)	39.96±0.17	37.84±0.30	38.13±1.74	36.33±0.39

Data are expressed as mean ± S.E.M. (n =5-10). (***) Denotes *P*<0.001 as compared to the SC/Vehicle. (*) Denotes *P*<0.05 as compared to the SC/Vehicle. ($^{\#\#\#}$) Denotes *P*<0.001 as compared to HPD/Vehicle. ($^{\#\#}$) Denotes *P*<0.01 as compared to HPD/Vehicle. SC= Standard Chow; HPD= Highly Palatable Diet.

Table 3. Effect of *E. uniflora* extract treatment in rats exposed to a highly palatable diet on oxidative stress parameters in the prefrontal cortex, hippocampus and striatum

Parameters	SC/Vehicle	SC/*E. uniflora*	HPD/Vehicle	HPD/*E. uniflora*
	Prefrontal cortex			
TBARS	0.99±0.13	1.14±0.15$^{\#\#}$	2.02±0.13***	1.26±0.13$^{\#\#}$
Total SH content	37.60±3.29	32.52±3.01	24.08±2.60*	33.17±2.81
CAT	1.41±0.10	1.28±0.11$^{\#\#}$	0.85±0.06***	1.16±0.01$^{\#}$
SOD	32.72±2.47	29.07±2.95	18.32±1.39***	26.16±1.35$^{\#}$
GPX	55.81±3.87	53.83±3.07	51.32±2.17	50.26±3.37
	Hippocampus			
TBARS	1.38±0.10	0.88±0.04*	2.26±0.16***	1.77±0.10$^{\#}$
Total SH content	30.04±2.35	28.95±1.44	20.87±0.95**	23.97±0.35
CAT	1.51±0.12	1.29±0.05	1.12±0.05**	1.57±0.06$^{\#\#\#}$
SOD	32.17±0.71	26.57±1.60	20.98±2.27***	27.62±0.64$^{\#}$
GPX	53.20±3.85	51.90±3.05	54.25±2.90	65.32±2.50
	Striatum			
TBARS	2.23±0.11	1.76±0.25$^{\#\#\#}$	3.50±0.31***	1.79±0.28$^{\#\#\#}$
Total SH content	34.28±0.93	28.00±1.33	23.11±1.26**	29.51±2.41
CAT	1.82±0.17	1.67±1.15	1.09±0.08**	1.68±0.16$^{\#}$
SOD	36.57±3.56	34.56±3.34	21.27±0.39***	35.78±2.36$^{\#}$
GPX	55.59±4.20	66.92±2.21	57.08±4.98	58.73±2.53

Data are expressed as mean ± S.E.M. (n =5-10). TBARS levels are reported as nmol TBARS per mg protein, thiol content as nmol TNB per mg protein, enzyme activities (CAT, SOD, GPX) as units/mg protein. (***) Denotes $P<0.001$ as compared to the SC/Vehicle. (**) Denotes $P<0.01$ as compared to the SC/Vehicle. (*) Denotes $P<0.05$ as compared to the SC/Vehicle. ($^{\#\#\#}$) $P<0.001$ as compared to the HPD/Vehicle. ($^{\#\#}$) $P<0.01$ as compared to the HPD/Vehicle. ($^{\#}$) $P<0.05$ as compared to the HPD/Vehicle. SC= Standard Chow; HPD= Highly Palatable Diet.

Table 4. Effect of *E. uniflora* extract treatment in rats exposed to a highly palatable diet on acetylcholinesterase activity in prefrontal cortex, hippocampus and striatum

AChE (µmol/h/mg protein)

	SC/Vehicle	SC/*E. uniflora*	HPD/Vehicle	HPD/*E. uniflora*
Prefrontal cortex	1.24± 0.118	1.03 ±0.102	1.66 ±0.091*	1.02 ± 0.066$^{###}$
Hippocampus	1.25 ± 0.012	1.35 ± 0.046	1.23 ± 0.087	1.47 ± 0.017
Striatum	6.07 ± 0.164	6.03± 0.010	5.66 ± 0.311	5.17 ± 0.195

Data are expressed as mean ± S.E.M. (n =4-6). (*) Denotes $P<0.05$ as compared to the SC/Vehicle. ($^{###}$) $P<0.001$ as compared to HPD/Vehicle. SC= Standard Chow; HPD= Highly Palatable Diet.

5. Artigo II

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Southern Brazilian native fruit shows neurochemical, metabolic and behavioral benefits in an animal model of metabolic syndrome

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Abstract

In this work, we evaluated the effects of *Psidium cattleianum* (Red Type) (PcRT) fruit extract on metabolic, behavioral, and neurochemical parameters in rats fed with a highly palatable diet (HPD) consisted of sucrose (65% carbohydrates being 34% from condensed milk, 8% from sucrose and 23% from starch, 25% protein and 10% fat). Animals were divided into 4 groups: standard chow, standard chow + PcRT extract (200 mg/Kg/day by gavage), HPD, HPD +extract. The animals were treated for 150 days. Concerning chemical profiling, LC/PDA/MS/MS analysis revealed cyanidin-3-*O*-glucoside as the only anthocyanin in the PcRT extract. Our results showed that the animals exposed to HPD presented glucose intolerance, increased weight gain and visceral fat, as well as higher serum levels of glucose, triacylglycerol, total cholesterol, LDL-cholesterol and interleukin-6. These alterations were prevented by PcRT. In addition, HPD caused an increase in immobility time in a forced swimming test and the fruit extract prevented this alteration, indicating an antidepressant-like effect. PcRT treatment also prevented increased acetylcholinesterase activity in the prefrontal cortex caused by HPD consumption. Moreover, PcRT extract was able to restore Ca^{2+}-ATPase activity in the prefrontal cortex, hippocampus, and striatum, as well as Na^{+},K^{+}-ATPase activity in the prefrontal cortex and hippocampus. PcRT treatment decreased thiobarbituric acid-reactive substances, nitrite, and reactive oxygen species levels and prevented the reduction of superoxide dismutase activity in all cerebral structures of the HPD group. Additionally, HPD decreased catalase in the hippocampus and striatum. However, the extract prevented this change in the hippocampus. Our results showed that this berry extract has antihyperglycemic and antihyperlipidemic effects, and neuroprotective properties, proving to be a potential therapeutic agent for individuals with metabolic syndrome.

Keywords: metabolic syndrome; highly palatable diet; *P. cattleianum*; natural products; phenolic compounds; neuroprotection

1. Introduction

Metabolic syndrome (MetS) is characterized by a combination of cardiovascular risk factors including hyperglycemia, dyslipidemia, and insulin resistance (IR) (Auberval et al. 2017). In addition, obesity has also been involved in this syndrome and may contribute to pro-inflammatory and oxidant effects (Wellen and Thompson 2010; Auberval et al. 2017). It has been shown that highly palatable diet (HPD) stimulates food intake and, consequently, promotes alterations in homeostasis related to utilization of glucose, and may lead to hyperglycemia, IR, and fatty acid oxidation (Erlanson-Albertsson 2005).

In normal metabolism, there is a balance between oxidants and antioxidants in order to maintain redox homeostasis. However, in increased energy intake, an imbalance can occur, leading to overproduction of reactive oxygen species (ROS) and reduction of antioxidant defenses. It can result in oxidative stress, which may contribute to the development of ailments such as coronary heart disease, neurological disorders, diabetes and MetS (Paredes-Lopez et al. 2010; Da Silva et al. 2014).

Evidence suggests that inflammation as well oxidative stress present in MetS can be related to the development of neuropsychiatric disorders such as depression, anxiety, schizophrenia and bipolar disorder, supporting the importance of the brain in the physiopathology of peripheral energy balance and glucose homeostasis (Farooqui et al. 2012; Zhang and Yoa 2013; Gancheva et al. 2017; Oliveira et al. 2017a; Oliveira et al. 2017b). Furthermore, increases in ROS can lead to inhibition of the enzyme Na^+, K^+-ATPase through oxidation of groups essential for enzyme activity (Srikanthan et al. 2016). The enzymes Na^+,

K^+-ATPase and Ca^{2+}-ATPase are transmembrane proteins, known not only for the function of ion pump, but also because they are important for cellular and synaptic functions (Kirshenbaum et al. 2011; Carvalho et al. 2015; Srikanthan et al. 2016; Lui et al. 2016). In addition, Srikanthan and collaborators (2016) showed that the activation signaling cascade of Na^+,K^+-ATPase contributes to the worsening of obesity, dyslipidemia, diabetes, and atherosclerosis.

Acetylcholinesterase (AChE) is an enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine (ACh) in cholinergic synapses (Zarros et al. 2009; Kade et al. 2013). The increase in the activity of this enzyme leads to a reduction in the efficiency of cholinergic neurotransmission due to a decrease in ACh levels, contributing to cognitive and neuropsychiatric disorders (Kade et al. 2013). In addition, an increase in AChE activity has been observed in animal models of diabetes and MetS, and oxidative stress seems to be involved in this increase, given that antioxidant therapies contribute to the maintenance of adequate levels of ACh in the synaptic cleft (Melo et al. 2003; Zarros et al. 2009; Kade et al. 2013; Oliveira et al. 2017b).

Given the variety of factors involved in the physiopathology of MetS and the use of multiple therapies, often with adverse effects, alternative therapies have been sought to prevent or control this syndrome. It has been shown that natural products have several bioactive compounds that exhibit antioxidant actions and may contribute to the prevention of diseases such as MetS, diabetes, and neuropsychiatric disorders (Devalaraja, et al. 2011). Complementarily, biological activity seems be result of the combination of several compounds and thus the isolation process may lead to its loss or reduction (Williamson 2001; Raskin and Ripoll 2004; Cravotto et al. 2010; Carmona and Pereira 2013).

Psidium cattleianum Sabine Red Type (*Myrtaceae*) is a native fruit of Brazil that has a wide variety of uses in popular medicine, and has hypoglycemic, antibacterial, anti-inflammatory, analgesic, and central nervous system activities (Mattos 1989; Inhwan et al. 2012). Additionally, these red fruits have a high content of phytochemicals such as, anthocyanins, flavonoids and phenolic acids, which also provide antioxidant properties that, can minimize oxidative damage caused by ROS (Biegelmeyer et al. 2011; Silva et al. 2014).

Studies carried out by our research group in order to seek new strategies for the control and prevention of MetS have already demonstrated that red fruit extracts such as *Eugenia uniflora* and *Vaccinium virgatum* present anti-hyperglycemic, anti-hyperlipidemic, and neuroprotective activities (Oliveira et al. 2017a; Oliveira et al. 2017b). In this sense, the objective of this study was to investigate whether *P. cattleianum* (PcRT) fruit extract could protect rats fed with a HPD against metabolic, neurochemical, and behavioral alterations observed in MetS.

2. Materials and Methods

2.1 Phytochemicals

2.1.1 Extraction

PcRT fruits were harvested in an orchard belonging to *Embrapa Clima Temperado* (Brazilian Agricultural Research Corporation) Pelotas/RS, Brazil. The extracts were prepared according to Bordignon et al. (2009). Unprocessed frozen PcRT fruits (30 g) were sonicated for 30 min at 25ºC in 90 mL 70:30 v/v ethanol-water. The pH of the solution was adjusted to 1.0. After this, the crude extracts

were filtered, the ethanol was evaporated under reduced pressure and the remaining aqueous solution was lyophilized yielding the test samples.

2.1.2 Total phenolic, flavonoid and anthocyanin contents

The total phenolic content was determined according to Singleton et al. (1999), and expressed as milligrams of gallic acid per 1 g of dried extract. The total flavonoid content was measured as described by Miliauskas et al. (2004) and results were expressed as milligrams of gallic acid per 1 g of dried extract. Anthocyanins were quantified as described by Lee et al. (2005) and reported as milligrams of cyanidin-3-glucoside per 1 g of dried extract. All data were expressed as means ± SD and analyses were performed in triplicate.

2.1.3 *Identification of anthocyanins by LC/PDA/MS/MS*

Anthocyanins were identified using an Acquity-UPLC^{TM} coupled to a photodiode array detector (PDA) and to a high-resolution mass spectrometer (Xevo® G2 QTof model – WATERS®, USA). Chromatographic separation was performed using a C18 chromatography column (Synergi^{TM}- Phenomenex®, USA) 4 µm, 150 x 2.0 mm at 40°C, and the injection volume was 5 µL. The elution was carried out using an aqueous solution of formic acid 2% (solvent A) and acetonitrile with 1% of formic acid (solvent B). The method used a linear gradient at constant flow rate (0.4 mL.min^{-1}). The total time of analysis was 34 min according to the following conditions: 0-10 min, 5-12% B; 10-29 min, 12-18% B; 29-33 min, 18% B; 33-34 min, 5% B. Detection was performed at 520 nm, and the range of spectral scanning in the visible region was 450 – 600 nm (PDA). Mass spectrometry was carried out with an electrospray ionization source (ESI)

set on negative ion mode, capillary voltage 1.0 kV, source block temperature 120°C, desolvation temperature 600°C; nebulizer nitrogen flow rate 80 L h^{-1}, desolvation nitrogen gas flow 800 L h^{-1}, and cone voltage, 40 V, controlled by MassLynx v.4.1 software for data acquisition and processing. The mass scanning ranged from 200 to 1500 m/z with a scan time of 0.5 s. MS/MS analysis was performed using a collision energy ramp (10 – 30 eV); and with argon as the collision gas. All analyses were performed in triplicate.

2.2 Animal and extract treatments

Wistar rats aged 21 days and maintained at 21-25^{o}C with free access to water and food, under a 12:12 h light:dark cycle (lights on at 7:00 a.m.) were used throughout this study. The animals were housed in groups of 3-4 per cage.

Forty male rats were divided into four groups: (1) standard chow group (SC) + vehicle, which received standard laboratory rat chow (50% carbohydrate from starch, 22% protein and 4% fat) and water; (2) SC + PcRT, with standard laboratory rat chow and PcRT; (3) HPD + vehicle, which received a diet consisted of sucrose (65% carbohydrates being 34% from condensed milk, 8% from sucrose and 23% from starch, 25% protein and 10% fat) and water (4) HPD + PcRT. The treatments lasted 150 days and the dose of extract administered was 200 mg/Kg/day by gavage. The animal model of MetS and the dose of PcRT extract were based on Oliveira et al. (2017a) and Oliveira et al. (2017b). The experiments were performed after approval by the Ethics Committee of the Institution (CEEA n° 9125) and all efforts were made to minimize animal suffering.

2.3 Body weight gain and food intake

Changes in body weight and food intake patterns of rats were measured throughout the experimental period. The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study. The quantity of food consumed by each group was recorded weekly, and the food consumption per rat was calculated for each group.

2.4 Sample collection and biochemical assay

After 150 days of food and extract administration, 24 h after last behavioral test and after 6 h of fasting the animals were euthanized by decapitation without anesthesia. At euthanasia, visceral fat was weighed and the blood collected. Serum was obtained by centrifugation at 800 x g (4°C) for 15 min. Prefrontal cortex (PFC), hippocampus (HP), and striatum (ST) were separated and stored at -80°C for subsequent biochemical analyses.

2.4.1 Glucose tolerance test

The glucose tolerance test was performed 2 days before the euthanasia. Rats were injected intraperitoneally with 50% glucose solution to achieve 2 mg/g of body weight. The glucose levels for all the groups were estimated using a glucometer (AccuChek Active, Roche Diagnostics®, USA) at 30, 60, and 120 min after the glucose injection by a small tail puncture to collect blood.

2.4.2 Serum biochemical parameters

Measurements of serum glucose, total cholesterol, cholesterol-LDL, cholesterol-HDL, triacylglycerol (TAG), urea, uric acid, and alanine

aminotransferase (ALT) levels were determined using commercially available diagnostic kits supplied by Labtest® (Labtest, MG, Brazil). Interleukin-6 (IL-6) quantity was assessed by ELISA using commercial kits (R&D Systems) according to the manufacturer's instructions.

2.5 Behavioral analysis

2.5.1 Open-field Test

Ambulatory behavior was assessed in an open-field test as previously described by Gazal, et al. (2015). The experimental apparatus consisted of a box measuring 40 x 60 x 50 cm. with the floor of the arena divided into 12 equal squares and placed in a sound-free room. Animals were placed in the rear left square and left to freely explore for 5 min during which time the number of squares crossed with all paws (crossing) was counted.

2.5.2 Forced Swimming Test (FST)

Depressive-like behavior was evaluated by the total duration of immobility in the forced swimming test, as previously described Huynh et al. (2011). Rats were individually forced to swim in an open cylindrical container 45 cm high and 20 cm in diameter and depth of 30 cm, with water at 22 ± 1°C and the total duration of immobility during a 5 min period was scored: rats were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep the head above water.

2.6 Tissue preparation

Prefrontal cortex (PFC), hippocampus (HP), and striatum (ST) were homogenized in sodium phosphate buffer, pH 7.4, containing KCl. The homogenates were centrifuged at 800 × *g* for 10 min at 4ºC and the supernatant was used for neurochemical analyses. Protein concentration was determined by the method of Lowry et al. (1951) or Bradford (1976).

2.7 Acetylcholinesterase (AChE) activity

AChE activity was determined as described in the colorimetric method of Ellman et al. (1961) with modifications and expressed as µmol AcSCh/h/mg of protein.

2.8 Determination of Ca^{2+}-ATPase and Na^{+},K^{+}-ATPase activities

Na^{+},K^{+}-ATPase and Ca^{+2}-ATPase activities were measured in the cerebral structures as described by Carvalho et al. 2015. The amount of inorganic phosphate (Pi) released was quantified colorimetrically, using KH_2PO_4 as the reference standard (Fiske and Subbarow 1927). The absorbance was measured at 630 nm.

The specific Na^{+},K^{+}-ATPase activity was calculated by subtracting the ouabain-insensitive activity from the overall activity (in the absence of ouabain) and was expressed in nmol of Pi/min/mg of protein. Different concentrations of ouabain were used to evaluate the activity of isoforms of Na^{+},K^{+}-ATPase. For this purpose, a classical pharmacological approach was used based on the isoform-specific sensitivity to ouabain (Niche et al.1999). The experiments were performed as previously described (Rambo et al. 2012). To determine if

treatments alter ouabain-sensitive ATPase activity, 3 µM or 4 mM ouabain was used (so as to only inhibit the Na^+,K^+-ATPase isoforms containing subunits α2/α3, or to inhibit all isoforms, respectively).

The Ca^{2+}-ATPase activity was determined by subtracting the activity measured in the presence of Ca^{2+} from that determined in the absence of Ca^{2+} (no added Ca^{2+} plus 0.1mM EGTA) and was expressed in nmol of Pi/min/mg of protein.

2.9 Oxidative stress parameters

2.9.1 Thiobarbituric acid-reactive substances (TBARS)

TBARS, a measure of lipid peroxidation, was determined according to the method described by Ohkawa et al. (1979) and reported as nmol TBARS/mg of protein.

2.9.2 Reactive oxygen species (ROS)

ROS formation was determined according to Ali et al. (1992). In this assay, the oxidation of dichloro-dihydro-fluorescein diacetate (DCFH-DA) to fluorescent dichlorofluorescein (DCF) was measured. DCF fluorescence intensity emission was recorded at 525 and 488 nm excitation 60 min after the addition of DCFH-DA to the medium. ROS levels were expressed as µmol DCF/mg of protein.

2.9.3 Nitrite assay

Nitrite concentrations were measured using the Griess reaction as described by Huang et al. 2009. For this reaction, 50 µL of sample was reacted with 50 µL Griess reagent for 10 min at room temperature. The absorbance was

measured at 540 nm using a 96-well microplate reader and the amount of nitrite in the supernatant was compared to a standard curve of known concentrations of sodium nitrate.

2.9.4 Total thiol content assay

This assay was performed as described by Aksenov and Markesbery (2001). Results were reported as nmol TNB/mg of protein.

2.9.5 Antioxidant enzyme activities

Catalase (CAT) activity was assayed according to Aebi (1984) based on the decomposition of H_2O_2. Superoxide dismutase (SOD) activity was measured by the method described by Misra and Fridovich (1972) which is based on the inhibition of superoxide-dependent adrenaline auto-oxidation. Glutathione peroxidase (GPx) activity was measured using a commercially available diagnostic kit supplied by (RANSEL®; Randox Lab, Antrim, United Kingdom). Enzyme activities were reported as units/mg of protein.

2.10 Statistical analysis

Measured values were expressed as means ± S.E.M. Glucose tolerance was analyzed by repeated measures ANOVA and Bonferroni's *post-hoc* correction. The interaction between groups was determined by two-way ANOVA followed by Bonferroni's *post-hoc* test. A value of $P \leq 0.05$ was considered to be statistically significant. Analyses were performed using the software GraphPad PRISM 5®.

3. Results

3.1 Phytochemical characterization

According to the phytochemical characterization results, the total phenolic content in PcRT was 16.72 ± 0.26 mg/g of dried extract. As to the total flavonoid content, 15.24 ± 2.09 mg/g of dried extract was detected. In addition, anthocyanin content was 2.48 ± 0.09 mg/g of dried extract. Analysis by LC/PDA/MS/MS showed only the presence of cyanidin-3-*O*-glucoside in the fruit extract captured at 515 nm and with a retention time of 8.49 min. The chemical structure of anthocyanin was identified by comparison with the MS/MS data of the standard samples. The anthocyanin mass and main fragmentation figures were $[M]^+$449.1065 *m/z* (error - ppm: -4.2) and 287 (*m/z*), respectively.

3.2 Metabolic status

As shown in Figure 1A, HPD induced impaired glucose tolerance ($P < 0.01$), and treatment with PcRT prevented this change in the animals submitted to the HPD. The same profile was observed in area-under-curve (A.U.C) analysis (Fig. 1B). Moreover, two-way ANOVA showed significant differences between the groups, suggesting that the PcRT treatment prevented the increase of some metabolic parameters such as weight gain [$F(1,25) = 11.63$; $P < 0.01$], visceral fat mass [$F(1,27) = 5.18$; $P < 0.05$], blood glucose levels [$F(1,15) = 5.12$; $P < 0.05$], total cholesterol [$F(1,21) = 25.79$; $P < 0.001$], cholesterol-LDL [$F(1,13) = 92.24$; $P < 0.001$], TAG [$F(1,13) = 6.34$; $P < 0.05$] and IL-6 [$F(1,16) = 68.39$; $P < 0.001$] caused by HPD. There were no significant differences between any of the groups tested for cholesterol-HDL, urea, uric acid, and ALT ($P > 0.05$) (Table 1).

3.3 Behavioral parameters

Two-way ANOVA showed that PcRT treatment prevented the increase in immobility time in the FST induced by consumption of HPD [$F(1,23) = 10.69$, $P < 0.01$] (Fig. 2A). In order to rule out nonspecific motor effects that could influence activity in the FST, rats were also submitted to an open-field test (Fig. 2B). Statistical analysis did not reveal an interaction between groups in this task [$F(1,24) = 2.00$ $P > 0.05$], suggesting that the effects of PcRT treatment in the FST are not related to changes in locomotor activity.

3.4 Neurochemical parameters

Table 2 shows that PcRT treatment prevented the increase in the AChE activity caused by consumption of HPD in the PFC since two-way ANOVA revealed interaction between the groups [$F(1,17) = 8.85$, $P < 0.01$]. However, this enzyme activity was not altered in the HP [$F(1,10) = 78.21$, $P > 0.05$] and ST [$F(1,13) = 1.53$, $P > 0.05$].

With regard to ATPase activity, two-way ANOVA showed a significant interaction between experimental groups, suggesting that PcRT prevented a decrease in the activity of Ca^{2+}-ATPase in PFC [$F(1,12) = 12.63$, $P < 0.01$], HP [$F(1,12) = 27.48$, $P < 0.001$] and ST [$F(1,11) = 25.60$, $P < 0.001$] induced by HPD (Fig. 3A-C).

The effect of fruit extract and HPD on the total Na^+,K^+-ATPase activity and its isoforms in the cerebral structures of rats is demonstrated in Fig 4. Two-way ANOVA revealed significant differences between the experimental groups, suggesting that the PcRT treatment prevented the decrease in total Na^+,K^+-ATPase [$F(1,14) = 7.85$, $P < 0.01$]), α2,3- [$F(1,12) = 9.48$, $P < 0.01$]) and α1-

isoform activities [$F(1,12)=14.93$, $P < 0.01$] caused by HPD consumption. In HP (Fig. 4D-F), the activity of total Na^+,K^+-ATPase [$F(1,13)=12.56$, $P < 0.01$] and α2,3-isoform [$F(1,12)=7.08$, $P < 0.05$] was reduced by HPD consumption, and PcRT was able to restore ion pump activities. However, statistical analysis (two-way ANOVA) did not show an interaction [$F(1,12)=1.20$, $P > 0.05$] in α1-Na^+,K^+-ATPase but a significant main effect of HPD [$F(1,12)=27.04$, $P < 0.001$]. Additionally, we did not observe significant differences in the activity of total Na^+,K^+-ATPase [$F(1,13) = 0.45$, $P > 0.05$], α2,3- [$F(1,13) = 0.95$, $P > 0.05$] and α1 isoforms [$F(1,13) = 0.69$, $P > 0.05$] in the ST (Fig. 4G-I).

Two-way ANOVA showed a significant interaction between experimental groups, suggesting the effect of PcRT administration on certain oxidative stress parameters in the cerebral structures of animals exposed to HPD. We observed that PcRT was able to prevent the increase in TBARS levels in HP [$F(1,17) = 9.53$, $P < 0.01$], ST [$F(1,22) = 4.47$, $P < 0.05$] and PFC [$F(1,21) = 10.23$, $P < 0.05$]. The same effect was observed for ROS levels in HP [$F(1,11) = 21.59$, $P < 0.001$], ST [$F(1,13) = 67.84$, $P < 0.001$] and PFC [$F(1,18) = 12.28$, $P < 0.01$]. The increase in nitrite levels was prevented by PcRT in HP [$F(1,13) = 13.11$, $P < 0.01$] and ST [$F(1,14) = 9.50$, $P < 0.01$]. However, although there is no interaction between groups, two-way ANOVA revealed that PcRT reduced the nitrite concentrations enhanced by HPD consumption in PFC (PcRT: [$F(1,15) = 10.47$, $P<0.01$], HPD: [$F(1,15) = 7.91$, $P<0.05$]). In contrast, two-way ANOVA did not show any significant differences in total thiol content in PFC [$F(1,19) = 0.48$, $P > 0.05$] and HP [$F(1,23) = 0.23$, $P > 0.05$]. In ST, no interaction between groups was demonstrated, but a significant main effect of HPD in total thiol content [$F(1,15) = 34.82$, $P < 0.001$] (Table 3) was recognized.

In addition, we evaluated the activity of antioxidant enzymes in brain structures, and two-way ANOVA showed that PcRT treatment prevented the diminution of SOD activity in PFC [$F(1,20) = 7.36$, $P < 0.05$], HP [$F(1,31) = 4.66$, $P < 0.05$] and ST [$F(1,22) = 9.38$, $P < 0.01$] caused by consumption of HPD. Moreover, PcRT treatment prevented the reduction of CAT activity caused by HPD in HP [$F(1,20) = 4.53$, $P < 0.05$]. However, statistical analysis did not show an interaction in ST, but a significant main effect of HPD [$F(1,19) = 5.77$, $P < 0.05$]. Furthermore, we did not observe any significant difference in the activity of cortical CAT [$F(1,18) = 1.73$, $P > 0.05$], or in GPx activity from PFC [$F(1,25) = 0.01$, $P > 0.05$], HP [$F(1,16) = 0.51$, $P > 0.05$] and ST [$F(1,16) = 0.60$, $P > 0.05$] (Table 3).

4. Discussion

The berries represent a variety of small fruits characterized by purple, blue or red color and contain high levels of polyphenols, including anthocyanins, flavonoids, and other phenolic compounds (Vendrame et al. 2016). The PcRT is a berry belonging to the family *Myrtaceae* and is popularly known to demonstrate important antioxidant, anti-inflammatory, and hypoglycemic action due to the presence of bioactive compounds such as polyphenols (Inhwan et al. 2012; Ribeiro et al. 2014). We detected a single anthocyanin in the extract, cyanidin-3-glycoside, widely found pigment responsible for the color of berries. This finding is relevant in a native berry from the south of Brazil since cyanidin-3-glucoside has been reported as responsible for several beneficial activities involving parameters linked to MetS (Bhaswant et al. 2015).

In the present work, we evaluated the effects of treatment with PcRT fruit extract on metabolic, neurochemical, and behavioral parameters in animals subjected to the MetS model induced by the consumption of HPD. PcRT extract was able to prevent some of the metabolic changes induced by HPD, such as weight gain and visceral fat accumulation, glucose intolerance, hyperglycemia, and dyslipidemia. In accordance, clinical and preclinical studies have shown a reduction of glycemic index and beneficial effects in lipid metabolism by guava, belonging to *Psidium* genus (Batista et al. 2018; Tey et al. 2017). It has been reported that increased TAG may cause reduction in peripheral insulin action (Khanal et al. 2012). In addition, it is likely that the weight gain observed in animals receiving HPD is related to increased visceral fat, since fat accumulation in this region has been reported as a determinant factor in the development of IR and MetS (Wellen and Thompson 2010; Khanal et al. 2012). Moreover, increased visceral fat leads to increased production of proinflammatory cytokines such as tumor necrosis factor (TNF-α), interleukin-1beta (IL-1β), C-reactive protein (CRP) and IL-6 (Bullo et al. 2007; Shah et al. 2008; Jayarathne et al. 2017). We observed that HPD increased IL-6 levels and PcRT extract was able to prevent this change. Furthermore, data in the literature have demonstrated that elevated levels of IL-6 have been associated with an increased risk of diabetes, obesity and neuropsychiatric disorders (Bullo et al. 2007; Rudolf et al. 2014; Jayarathne et al. 2017).

Bioactive compounds present in PcRT fruits such as phenolic compounds have an important effect on insulin signaling as well as on the reduction of weight, glucose, TAG, total cholesterol and LDL-cholesterol (Collins et al. 2015; Bhaswant et al. 2015; Vendrame et al. 2016). The mechanisms underlying the

actions of polyphenols present in *P. cattleianum* fruit extract are not well elucidated; however, some studies suggest that these compounds may inhibit digestive enzymes such as α-amylase and the absorption of glucose in the intestine (Hanhineva et al. 2010). Polyphenols such as quercetin and phenolic acids also act on the inhibition of sodium dependent glucose (SGLT1) and GLUT2 transporters. Inhibition of these glucose transporters could attenuate glucose level excursions after a meal (Manzano and Williamson 2010). Furthermore, some studies have reported that cyanidin-3-glycoside may increase fatty acid oxidation through AMP-activated protein kinase (AMPK) signaling. Activation of AMPK leads to the phosphorylation and inactivation of acetyl-CoA carboxylase, increasing fatty acid oxidation, and leading to decreased visceral fat and improved glucose metabolism (Guo et al. 2012; Bhaswant et al. 2015).

It is known that polyphenols present in the fruits of PcRT have important antioxidant and anti-inflammatory actions (Madani et al. 2015; Jayarathne et al. 2017). In addition, dietary interventions with compounds such as anthocyanins and flavonoids may modulate several systems that likely improve glucose homeostasis and insulin sensitivity (Bagul et al. 2012; Vendrame et al. 2016). In this sense, we observed that the consumption of HPD increased the levels of ROS, TBARS, and nitrite levels in the brain tissues tested, and that the treatment with PcRT was able to prevent the damage caused by HPD. We also demonstrated a reduction of thiol content in ST of animals exposed to HPD. Corroborating our findings, some data demonstrated that an increase of energy intake might lead to enhanced ROS in metabolically-active tissues such as the brain, causing lipid peroxidation and increased glucose oxidation (Madani et al. 2015; Trevino et al. 2015). It is also known that the brain uses a significant

amount of oxygen and adenosine triphosphate resulting in a high susceptibility to oxidative stress (Halliwell and Gutteridge 2007). In addition, high nitric oxide levels, both in animals and in diabetic humans, can induce oxidative damage through the formation of peroxynitrite (Bagul et al. 2012). We also evaluated the activity of the antioxidant enzymes SOD, CAT, and GPx and verified that the consumption of HPD was able to decrease the activity of the antioxidant enzyme SOD in all tissues studied. However, treatment with PcRT was able to prevent this reduction. Furthermore, we observed that HPD consumption decreased the activity of the antioxidant enzyme CAT only in the ST and HP of the animals tested. In contrast, treatment with PcRT extract prevented the decrease in the activity of this enzyme only in the HP of the animals tested. Data in the literature also demonstrate a significant decrease in antioxidant enzymes such as SOD and CAT in brain tissues such as PFC, HP, and ST of animals fed HPD (Trevino et al. 2015; Oliveira et al. 2017a; Oliveira et al. 2017b).

Recently, it has been suggested that MetS may cause neuropsychiatric complications such as depression, since chronic hyperglycemia and IR can trigger an inflammatory state and oxidative stress inducing neurotransmitter oxidation, neuroinflammation and neural death (Farooqui et al. 2012; Trevino et al. 2015; Oliveira et al. 2017a; Oliveira et al. 2017b; Gancheva et al. 2017). Hence, we evaluated the depressive-like behavior of animals that received HPD and observed that the consumption of this diet increased the immobility time in the FST indicating a depressive-like behavior. However, treatment with PcRT extract prevented this alteration. Corroborating our findings, some studies have demonstrated an antidepressant-like effect of certain berries (Kumar et al. 2012; Oliveira et al. 2017a; Oliveira et al. 2017b). In addition, Gancheva et al. (2017)

also demonstrated an increase in the time of immobility in FST of animals submitted to a diet enriched with saturated fat and fructose, indicating a depressive-like behavior, which in turn correlated with the metabolic effect of diet, especially with lipid peroxidation. Moreover, it has been suggested that the increase in the production of pro-inflammatory cytokines mainly produced by visceral adipose tissue has shown a positive correlation between obesity and neuropsychiatric disorders (Ambrósio et al., 2018). In fact, in our study we observed an increase in serum levels of IL-6 in animals submitted to HPD.

In an attempt to clarify the behavioral changes found in our study, we evaluated the influence of HPD and PcRT on the activity of Na^+,K^+-ATPase and its α1, α2, and α3 isoforms in the PFC, HP, and ST. Thus, we observed that the consumption of HPD significantly decreased the activity of this enzyme in PFC and HP and that the treatment with PcRT was able to restore enzyme activity. Corroborating our findings, Gamaro et al. (2003) and Quines et al. (2016) have also demonstrated an association between the reduction of Na^+,K^+-ATPase and the increase of FST immobility time in animals submitted to a model of depression, reinforcing the hypothesis that changes in the activity of this enzyme may be related to behavioral alterations. Besides, Na^+,K^+-ATPase is responsible for maintaining the cellular electrochemical balance, which is fundamental in the differentiation of the nervous system. However, a decrease in its catalytic activity directly compromises neural function (Carvalho et al. 2015; Quines et al. 2016).

Ca^{2+}-ATPase is also an important enzyme and plays a role in maintaining electrolyte balance in all mammals. This enzyme regulates the concentration of intracellular Ca^{2+} since its accumulation can damage the cell (Carvalho et al. 2015). In this regard, we observed that the consumption of HPD decreased

enzymatic activity in all tissues tested. On the other hand, treatment with PcRT prevented this decrease. The neuroprotective effect of the PcRT extract may be related to its antioxidant activity, since these enzymes are involved in the maintenance of the ionic gradient across the cell membrane and, therefore, its inhibition can be attributed to cell membrane lipoperoxidation and ROS increase, which may lead to the oxidation of groups essential for enzymatic activity (Sharma et al. 2009; Carvalho et al. 2015; Srikanthan et al. 2016). Carvalho et al. (2015) also related the decrease in Ca^{2+}-ATPase and Na^{+},K^{+}-ATPase activity to the increase of oxidative stress markers. Data in the literature have demonstrated significant increases in AChE activity in brain structures of animals with metabolic diseases and dysfunctions in the central nervous system (Zarros et al. 2009; Liapi et al. 2010; Oliveira et al. 2017b). We can speculate that the increase in this enzyme activity observed in PFC of the animals exposed to HPD may be related to a disturbance in Ca^{2+} homeostasis, since the influx of this ion leads to an increase in ROS production and, consequently, lipid peroxidation, which can cause a change in the conformational state of the enzyme (Melo et al. 2003). This fact would also explain the prevention of increased AChE activity by treatment with PcRT extract which has shown antioxidant effects.

In summary, our findings demonstrate that treatment with PcRT extract prevented the development of MetS induced by HPD, since it improved the body composition and demonstrated anti-hyperglycemic and anti-hyperlipidemic effects. In addition, the extract prevented the development of behavioral and neurochemical changes observed in this pathology. Thus, we can suggest that PcRT extract has neuroprotective properties, proving to be a potential therapeutic agent for individuals with MetS (Fig. 5).

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Conflict of interest

The authors declare that there are no conflicts of interest in this study

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Figure captions

Figure 1 Glucose tolerance test measured at the baseline (0), 30, 60 and 120 min after glucose injection (2 mg/g body weight) (A) and the respective area-under-curve (B). Data are expressed as mean ± S.E.M. (n=5 for group). (***) $P<0.001$ compared with vehicle/SC. (**) $P<0.01$ compared with vehicle/SC. ($^{###}$) $P<0.001$ when compared with vehicle/HPD. ($^{##}$) $P<0.01$ when compared with vehicle/HPD. Repeated measures ANOVA and one-way ANOVA followed by Bonferroni *post-hoc* test. SC = Standard Chow; HPD = Highly Palatable Diet.

Figure 2 Effect of *P. cattleianum* (red type) fruit extract treatment in rats exposed to a highly palatable diet on ambulatory behavior in immobility time in the FST (A) and open-field test (B). Results are expressed as mean ± S.E.M. (n=9-10 for group). (**) $P<0.01$ when compared with vehicle/SC. ($^{#}$) $P<0.05$ when compared with vehicle/HPD. Two-way ANOVA followed by Bonferroni *post-hoc* test. SC = Standard Chow; HPD= Highly Palatable Diet.

Figure 3 Effect of *P. cattleianum* (red type) fruit extract treatment in rats exposed to a highly palatable diet on Ca^{2+}-ATPase activity in prefrontal cortex (A), hippocampus (B) and striatum (C). Results are expressed as mean ± S.E.M. (n=5-6 for group). (***) $P<0.001$ when compared with vehicle/SC. (**) $P<0.01$ when compared with vehicle/SC. ($^{###}$) $P<0.001$ when compared with vehicle/HPD. ($^{##}$) $P<0.01$ when compared with vehicle/HPD. Two-way ANOVA followed by Bonferroni *post-hoc* test. SC = Standard Chow; HPD= Highly Palatable Diet.

Figure 4 Effect of *P. cattleianum* (red type) fruit extract treatment in rats exposed to a highly palatable diet on total Na^+,K^+-ATPase, α2,3- and α1-isoform activities in the prefrontal cortex, hippocampus and striatum. Results are expressed as mean ± S.E.M. (n=5-6 for group). (***) *P*<0.001 when compared with vehicle/SC. (**) *P*<0.01 when compared with vehicle/SC. (*) *P*<0.05 when compared with vehicle/SC. ($^{###}$) *P*<0.001 when compared with vehicle/HPD. ($^{##}$) *P*<0.01 when compared with vehicle/HPD. ($^{#}$) *P*<0.05 when compared with vehicle/HPD. Two-way ANOVA followed by Bonferroni *post-hoc* test. SC = Standard Chow; HPD= Highly Palatable Diet.

Figure 5 Overview of *P. cattleianum* (red type) fruit benefits on the metabolic, behavioral and neurochemical alterations induced in an experimental model of metabolic syndrome.

Figure 1

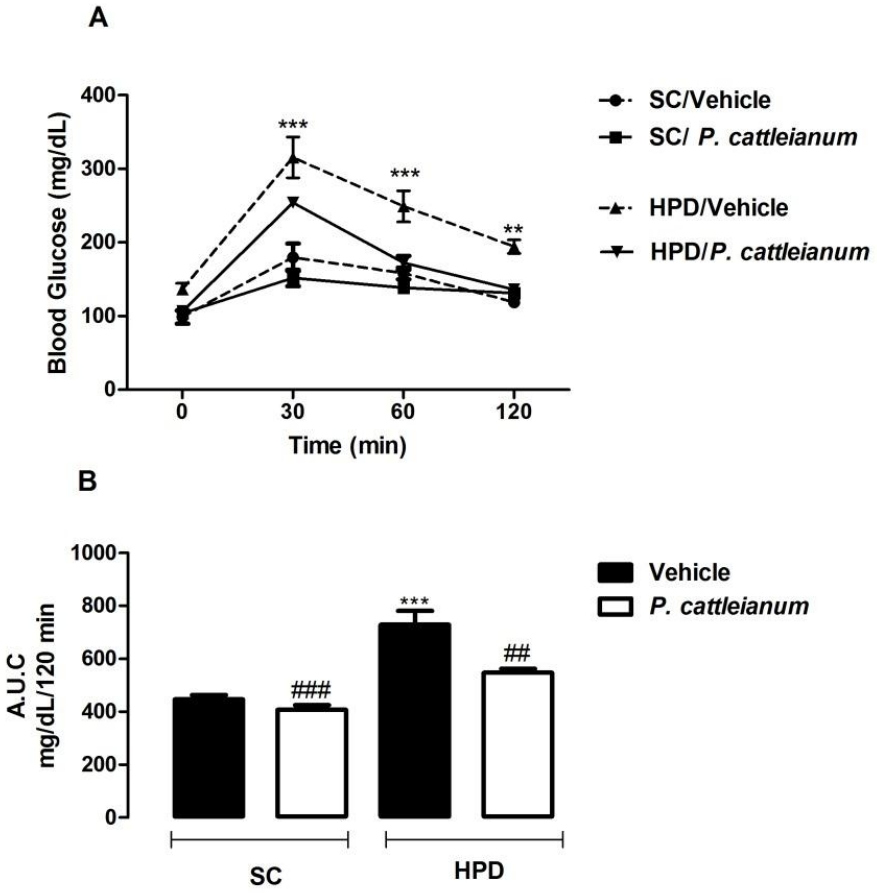
A
Blood Glucose (mg/dL)
400
300
200
100
0
0
30
60
120
Time (min)
***
***
**
SC/Vehicle
SC/ P. cattleianum
HPD/Vehicle
HPD/P. cattleianum
B
A.U.C
mg/dL/120 min
1000
800
600
400
200
0
###
***
##
SC
HPD
Vehicle
P. cattleianum

Figure 2

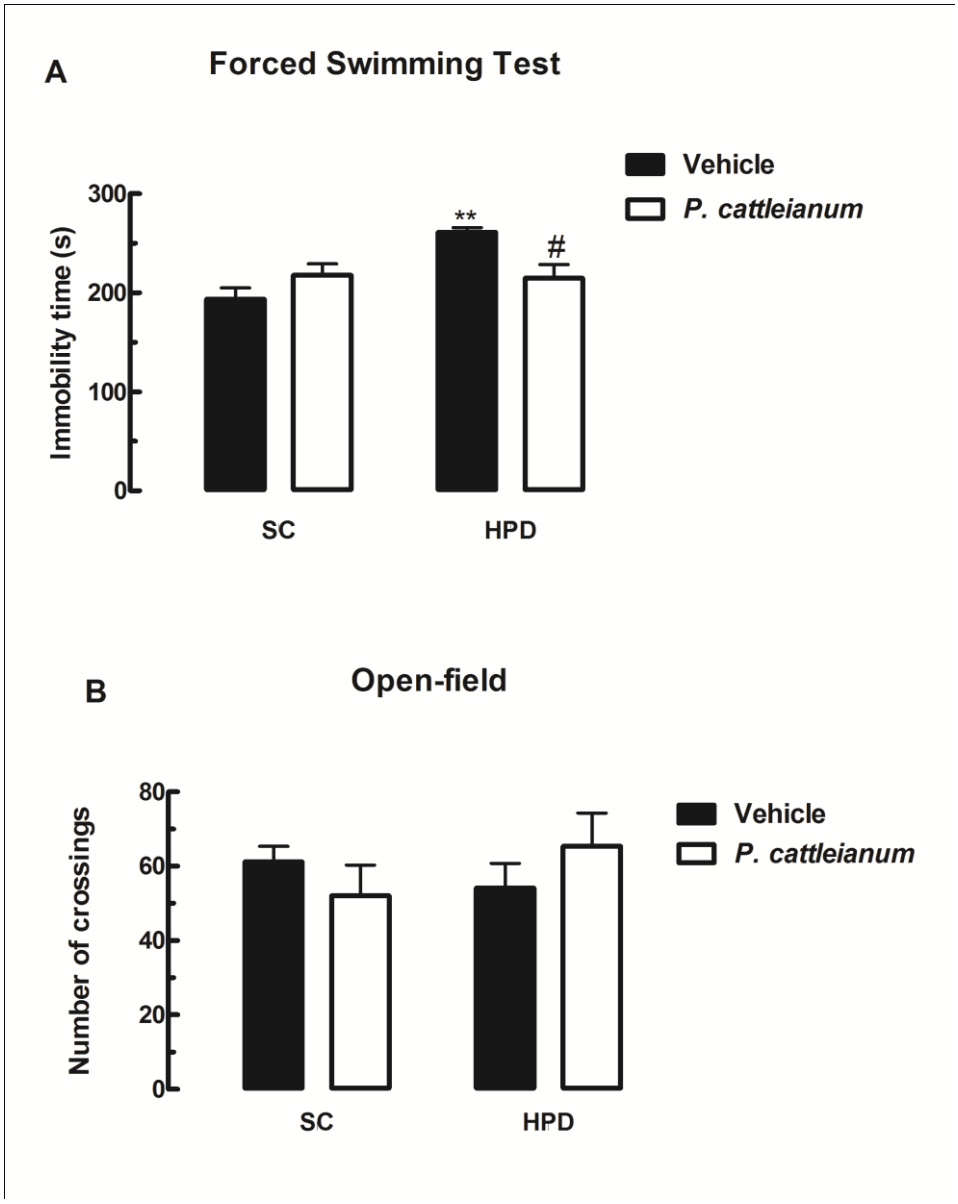
A
Forced Swimming Test
Vehicle
P. cattleianum
Immobility time (s)
300
200
100
0
**
#
SC
HPD
B
Open-field
Number of crossings
80
60
40
20
0
Vehicle
P. cattleianum
SC
HPD

Figure 3

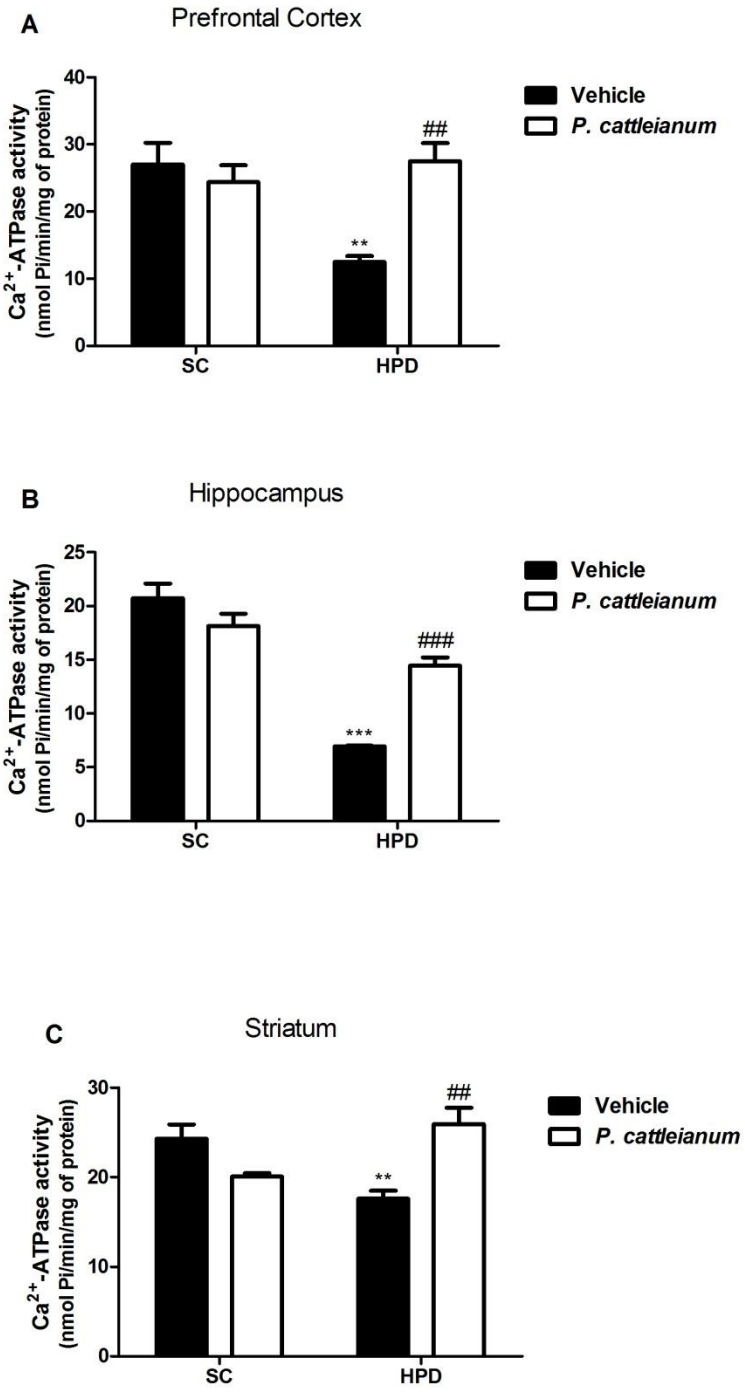
A
Prefrontal Cortex
Ca2+-ATPase activity (nmol Pi/min/mg of protein)
40
30
20
10
0
**
##
SC
HPD
Vehicle
P. cattleianum
B
Hippocampus
Ca2+-ATPase activity (nmol Pi/min/mg of protein)
25
20
15
10
5
0
***
###
SC
HPD
Vehicle
P. cattleianum
C
Striatum
Ca2+-ATPase activity (nmol Pi/min/mg of protein)
30
20
10
0
**
##
SC
HPD
Vehicle
P. cattleianum

Figure 4

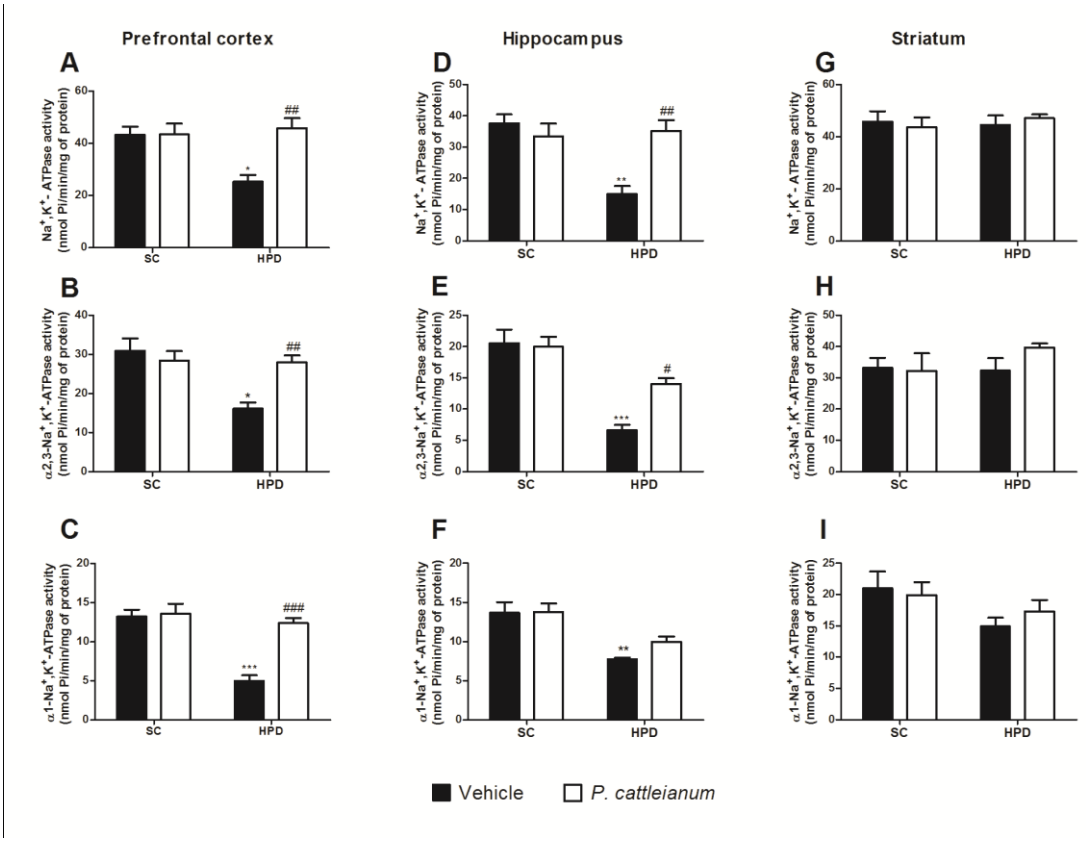
Prefrontal cortex
Hippocampus
Striatum
A
B
C
D
E
F
G
H
I
Na+,K+- ATPase activity (nmol Pi/min/mg of protein)
α2,3-Na+,K+-ATPase activity (nmol Pi/min/mg of protein)
α1-Na+,K+-ATPase activity (nmol Pi/min/mg of protein)
SC
HPD
Vehicle
P. cattleianum

Figure 5

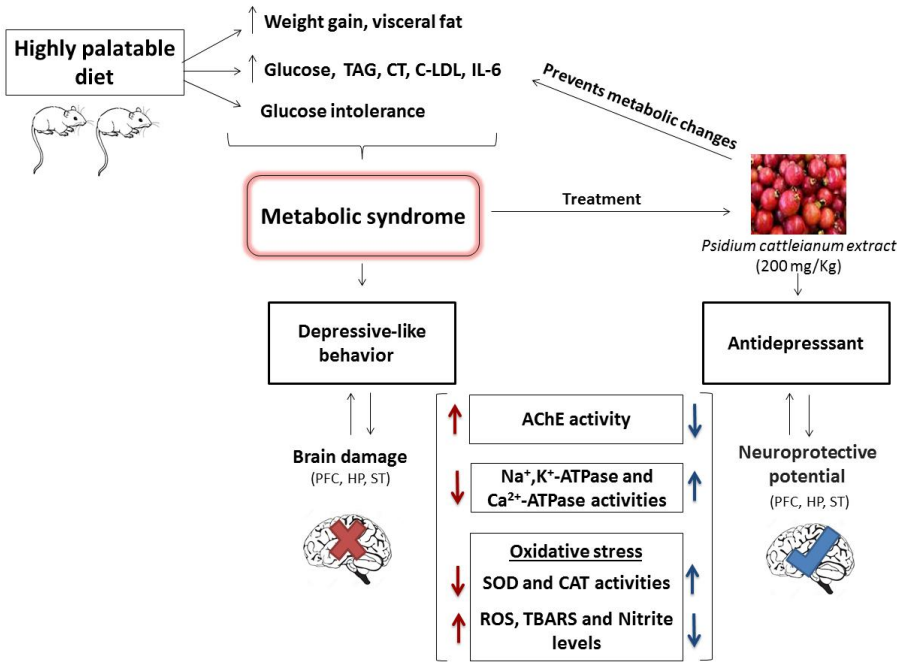
Highly palatable diet
Weight gain, visceral fat
Glucose, TAG, CT, C-LDL, IL-6
Glucose intolerance
Prevents metabolic changes
Metabolic syndrome
Treatment
Psidium cattleianum extract
(200 mg/Kg)
Depressive-like behavior
Antidepresssant
Brain damage
(PFC, HP, ST)
AChE activity
Na+,K+-ATPase and Ca2+-ATPase activities
Oxidative stress
SOD and CAT activities
ROS, TBARS and Nitrite levels
Neuroprotective potential
(PFC, HP, ST)

Table 1. Effect of *P. cattleianum* (red type) fruit extract treatment in rats exposed to a highly palatable diet on metabolic parameters

	SC/Vehicle	SC/*P. cattleianum*	HPD/Vehicle	HPD/*P. cattleianum*
Weight gain (g)	373±21.35	394±11.70$^{\#\#}$	475±19.64**	389±5.85$^{\#\#}$
Visceral fat mass (g)	15.66±1.85	11.33±1.74$^{\#\#\#}$	38.33±4.92***	20.89±1.07$^{\#\#\#}$
Glucose (mg/dL)	77.40±3.79	77.26±1.09$^{\#\#\#}$	131.73±7.11***	103.64±2.18$^{\#}$
Total cholesterol (mg/dL)	105.91±3.87	100.17±2.27$^{\#\#\#}$	156.85±5.71***	103.99±1.13$^{\#\#\#}$
LDL-cholesterol (mg/dL)	46.77±2.53	41.05±5.34$^{\#\#\#}$	100.30±2.82***	31.21±1.18$^{\#\#\#}$
HDL-cholesterol (mg/dL)	49.47±1.95	55.09±5.30	43.37±3.47	51.18±0.69
Triacylglycerol (mg/dL)	58.62±7.16	21.81±5.82**	98.00±3.20**	29.54±8.82$^{\#\#\#}$
Urea (mg/dL)	66.18±3.73	64.61±3.85	53.32±2.53	52.86±3.06
Uric acid (mg/dL)	1.01±0.09	1.05±0.09	0.68 ±0.10	0.98±0.05
ALT (U/mL)	39.96±0.17	37.19±0.29	36.13±0.88	37.25±0.45
IL-6 (pg/mg protein)	0.76±0.04	0.71±0.01	1.35±0.05***	0.60±0.06$^{\#\#\#}$

Data are expressed as mean ± S.E.M. (n =5-8). (***) *P*<0.001 as compared with the SC/Vehicle. (**) *P*<0.01 as compared with SC/Vehicle. ($^{\#\#\#}$) *P*<0.001 as compared with HPD/Vehicle. ($^{\#}$) *P*<0.05 as compared with HPD/Vehicle. SC= Standard Chow; HPD= Highly Palatable Diet. Two-way ANOVA followed by Bonferroni *post-hoc* test.

Table 2. Effect of *P. cattleianum* (red type) extract treatment in rats exposed to a highly palatable diet on acetylcholinesterase (AChE) activity in the prefrontal cortex, hippocampus and striatum

AChE (µmol AcSCh/h/mg of protein)

	SC/Vehicle	SC/ *P. cattleianum*	HPD/Vehicle	HPD/ *P. cattleianum*
Prefrontal cortex	1.24 ± 0.118	1.13 ± 0.036	1.71 ± 0.091*	1.04 ± 0.104$^{###}$
Hippocampus	1.25 ± 0.012	1.32 ± 0.043	1.17 ± 0.087	1.35 ± 0.043
Striatum	6.07 ± 0.164	6.30± 0.236	5.37 ± 0.463	5.01 ± 0.079

Data are expressed as mean ± S.E.M. (n =4-6). (*) $P<0.05$ as compared with SC/Vehicle. ($^{###}$) $P<0.001$ as compared with HPD/Vehicle. SC= Standard Chow; HPD= Highly Palatable Diet. Two-way ANOVA followed by Bonferroni *post-hoc* test.

Table 3. Effect of *P. cattleianum* (red type) extract treatment in rats exposed to a highly palatable diet on oxidative stress parameters in the cerebral structures

Prefrontal cortex				
Parameters	SC/Vehicle	SC/*P. cattleianum*	HPD/Vehicle	HPD/*P. cattleianum*
TBARS levels	0.99±0.13	1.05±0.07	1.83±0.15***	1.11±0.08$^{\#\#}$
Total SH content	30.44±3.72	25.92±1.48	21.06±2.33	20.79±3.28
ROS levels	46.20±4.15	47.76±3.72$^{\#\#}$	78.51±4.81***	48.03±4.46$^{\#\#\#}$
Nitrite levels	0.66±0.02	0.55±0.11	1.07±0.13**	0.63±0.05$^{\#\#}$
CAT activity	1.42±0.11	1.20±0.19	1.02±0.10	1.13±0.06
SOD activity	29.26±2.84	26.61±0.49	18.66±1.45**	26.18±1.25$^{\#}$
GPx activity	57.76±3.79	53.83±3.07	51.78±2.38	48.46±3.29
Hippocampus				
TBARS levels	1.38±0.10	1.46±0.09	2.50±0.18***	1.88±0.04$^{\#\#}$
Total SH content	28.40±1.93	30.04±3.00	20.87±0.95	20.50±0.91
ROS levels	51.64±3.11	57.13±2.15$^{\#\#\#}$	107.86±11.69***	51.04±2.46$^{\#\#\#}$
Nitrite levels	0.75±0.03	0.78±0.04	0.99±0.03**	0.71±0.07$^{\#\#}$
CAT activity	1.60±0.12	1.65±0.06	1.05±0.01**	1.49±0.09$^{\#\#}$
SOD activity	32.17±0.71	32.78±1.80	21.75±2.15**	30.57±2.04$^{\#\#}$
GPx activity	61.51±8.02	68.15±3.64	58.29±4.61	75.30±7.81
Striatum				
TBARS levels	2.23±0.11	1.87±0.26	3.50±0.25***	2.30±0.10$^{\#\#}$
Total SH content	34.28±0.93	29.80±0.52	23.11±1.26*	21.81±2.58
ROS levels	36.07±2.14	26.58±1.08*	87.79±2.58***	44.28±1.99$^{\#\#\#}$
Nitrite levels	0.58±0.07	0.69±0.04	0.98±0.06***	0.72±0.02$^{\#}$
CAT activity	1.74±0.20	1.67±0.11	1.11±0.11*	1.60±0.14
SOD activity	33.82±4.00	28.87±1.57	22.29±1.06*	32.77±2.60$^{\#}$
GPx activity	72.64±6.95	79.58±3.95	87.43±10.23	82.29±5.20

Data are expressed as mean ± S.E.M. (n =5-10). TBARS levels are reported as nmol TBARS/ mg of protein, thiol content as nmol TNB/ mg of protein, ROS levels as µmol DCF/mg of protein, nitrite levels as µmol/mg of protein, enzyme activities (CAT, SOD, GPx) as units/mg of protein. (***) P<0.001 as compared with SC/Vehicle. (**) P<0.01 as compared with SC/Vehicle. (*) P<0.05 as compared with SC/Vehicle. ($^{\#\#\#}$) P<0.001 as compared with HPD/Vehicle. ($^{\#\#}$) P<0.01 as compared with HPD/Vehicle. ($^{\#}$) P<0.05 as compared with HPD/Vehicle. SC= Standard Chow; HPD= Highly Palatable Diet. Two-way ANOVA followed by Bonferroni *post-hoc* test.

6. Manuscrito

Manuscrito a ser submetido

Brazilian native fruit extracts as modulators of purinergic and cholinergic signaling in blood cells and serum in a rat model of metabolic syndrome

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Abstract

The aim of this study was to evaluate the effects of red native fruit hydroalcoholic extract on the changes in metabolic, inflammatory, and thromboregulatory parameters in lymphocytes, platelets, and serum from rats with metabolic syndrome (MetS) induced by highly palatable diet (HPD) consumption. Wistar male rats aged 21 days were treated for 150 days and divided into 4 experimental groups: standard chow (SC), HPD, HPD and *Psidium cattleianum* extract (200 mg/kg/day by gavage), and HPD and *Eugenia uniflora* extract (200 mg/kg/day by gavage). Our results showed that rats exposed to HPD exhibited increased weight and visceral fat, and serum levels of glucose, triacylglycerol, total cholesterol, LDL-cholesterol, and interleukin-6 (IL-6). These alterations were prevented by administration of the extracts. Rats fed with HPD showed a decrease in NTPDase activity in lymphocytes and platelets and a decrease in 5´-nucleotidase levels in the platelets. Treatment with both extracts prevented these changes. Moreover, an increase in adenosine deaminase activity was observed in lymphocytes and serum of rats exposed to HPD. However, treatment with *E. uniflora* extract prevented this increase. In addition, administration of the fruit extracts prevented the increase in the activity of acetylcholinesterase in lymphocytes and butyrylcholinesterase in serum induced by the HPD. Fruit extracts of *E. uniflora* and *P. cattleianum* had anti-inflammatory and antithrombotic effects, modulating ectoenzyme and cholinesterase activities. Therefore, the results of this study demonstrate the therapeutic potential of Brazilian native fruit extracts in the prevention of complications associated with MetS.

Keywords: highly palatable diet; natural products; inflammation; antithrombotic; *E. uniflora; P. cattleianum.*

1. Introduction

MetS is characterized by a variety of cardiovascular risk factors, including hyperglycemia, dyslipidemia, hypertension, and visceral obesity [1]. However, visceral fat and insulin resistance (IR) are major risk factors for the development of inflammation in MetS [2, 3]. Increased visceral fat content leads to the recruitment of immune cells into adipose tissue, contributing to local and systemic inflammation [3]. In addition, dysfunction of this tissue increases the synthesis of molecules with pro-inflammatory actions, such as tumor necrosis factor (TNF-α), interleukin-1beta (IL-1β), interleukin-6 (IL-6), and protein C-reactive protein (CRP) [4, 5, 6]. IL-6 controls the production of CRP, which in turn induces the synthesis of other cytokines, such as the vascular cell adhesion molecule (VCAM) or the soluble intracellular adhesion molecule (sICAM) [4, 6]. The increase of these cytokines, when associated, has demonstrated a possible causative link between inflammation and atherosclerosis [4, 6].

It is already established that the nucleotides and nucleosides of adenine, such as adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP) and adenosine, act as extracellular messengers modulating the immune and inflammatory response [7]. However, alterations in these molecules may be involved in the pathophysiology of cardiovascular diseases as well as contribute to the development of type 2 diabetes, dyslipidemia, and MetS [7]. Ectonucleotidases, responsible for the hydrolysis and control of these molecules in extra cellular milieu, are expressed on the surface of several cells, including platelets and lymphocytes [8]. ATP is a pro-inflammatory molecule that stimulates lymphocytes and the release of pro-inflammatory. On the other hand, adenosine may inhibit platelet aggregation as

well as exhibit anti-inflammatory and immunosuppressive actions [7, 8]. Consequently, the enzymes E-NTPDase and adenosine deaminase (ADA) play important roles in purinergic modulation contributing to changes in the inflammatory and thromboregulatory processes [7, 8].

Acetylcholine (ACh) is also an important molecule with anti-inflammatory action that is involved in the regulation of immune functions [9]. This effect is related to the activity of the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), given that the increase in the activity of these enzymes may be associated with the different factors involved in the pathogenesis of MetS, such as IR, inflammation, and regulation of adipose tissue function [9-11].

Natural products appear to have therapeutic potential in the prevention of complications associated with MetS because flavonoids, such as anthocyanins, present in these compounds have important biological functions [12-14]. *P. cattleianum* and *E. uniflora* are native Brazilian fruits belonging to the family Myrtaceae [15, 16]. These fruits have demonstrated several beneficial such as antioxidative, anti-inflammatory, antihyperglycemic, antihyperlipidemic, and neuroprotective effects [15-19]. Therefore, the objective of this work was to evaluate the effects of the *hydroalcoholic* extracts of these native fruits on the changes in metabolic, inflammatory, and thromboregulatory parameters in platelets, serum, and lymphocytes of rats with MetS induced by HPD consumption.

2. Materials and Methods

2.1 Fruit extracts

P. cattleianum and *E. uniflora* fruits were harvested in an orchard belonging to *Embrapa Clima Temperado* (Brazilian Agricultural Research Corporation) Pelotas/RS, Brazil and *hydroalcoholic* extracts were prepared according to the method described by Bordignon et al. (2009) [20]. The phytochemical characterization of the extracts has been described in previous studies by our research group [18, 19]. LC/PDA/MS/MS analysis demonstrated that cyanidin-3-*O*-glucoside was the major anthocyanin present in *E. uniflora* fruit [18] and the only one identified in *P. cattleianum* [19].

2.2 Animals and treatment with the extracts

Male Wistar rats aged 21 days rats were obtained from the Central Animal House of the Federal University of Pelotas, Pelotas, RS, Brazil. The animals were maintained at 21-25°C with free access to water and food, under a 12:12 h light: dark cycle (lights on at 7:00 a.m.). The experiments were performed after approval by the Ethics Committee of Animal Experimentation of the institution (CEEA n° 9125).

Forty male rats were divided into four groups: (1) SC + vehicle, which received standard laboratory rat chow (50% carbohydrate from starch, 22% protein and 4% fat) and water; (2) HPD group + vehicle, which received an enriched sucrose diet (65% carbohydrates - 34% being from condensed milk, 8% from sucrose and 23% from starch, 25% protein and 10% fat) and water orally, (3) HPD + *P. cattleianum*, (4) HPD + *E. uniflora*. The treatments lasted 150 d and the dose of both extracts administered was 200 mg/kg/d by gavage (Fig. 1). The

animal model of MetS and the dose of the extract administered were based on previous studies [18, 19, 21].

2.3 Body weight gain and food intake

Changes in body weight and food intake patterns of rats were measured throughout the experimental period. The weight of each rat was recorded on d 0 and at weekly intervals throughout the course of the study. The quantity of food consumed by each group was recorded weekly, and the food consumption per rat was calculated for each group.

2.4 Sample collection and biochemical assay

After 150 days of food and extract administration and after 6 h of fasting, the animals were euthanized, visceral fat was weighed, and blood was collected.

2.4.1 Serum Preparation

The blood samples were collected in tubes without anticoagulant, and the samples were subsequently centrifuged at 2500×*g* for 15 min. The clot was removed and the serum obtained was stored at -80°C and used for biochemical analyses.

2.4.2 Serum biochemical parameters and IL-6 determination

Serum glucose, total cholesterol, cholesterol-LDL, and triacylglycerol (TAG) levels were determined using commercially available diagnostic kits supplied by Labtest® (Labtest, MG, Brazil). The IL-6 level was assessed by

ELISA using commercial kits (R&D Systems) according to the manufacturer's instructions.

2.4.3 Isolation of lymphocytes and platelets

Blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA) anticoagulant, and lymphocytes were separated by Ficoll-Histopaque density gradient separation as described by Böyum (1968) [22]. This methodology was employed for separating mononuclear cells; however, in the study performed by Jaques et al. (2011) [23], it was demonstrated that there is a high incidence of lymphocytes (approximately 95%) in these samples and the amount of monocytes is practically insignificant. Therefore, we treated the samples as lymphocyte preparations.

Platelet-rich plasma (PRP) was prepared as per the method of Pilla et al. (1996) [24] and modifications by Lunkes et al. (2004) [25].

2.5 Enzymatic assays using lymphocyte and platelet preparations

The activity of NTPDase in lymphocytes was determined according to Leal et al. (2005) [26] and in platelets according to Pilla et al. (1996) [24] and expressed in μmol Pi released/min/mg protein. The activity of adenosine deaminase in lymphocytes and platelets was determined according to Guisti and Galanti (1984) [27] with modifications. The results are expressed as U/L, where one unit (1 U) of adenosine deaminase is defined as the amount of enzyme required to release 1 mmol of ammonia per minute from adenosine under standard conditions.

AChE activity in lymphocytes was determined as described by Ellman et al. (1961) [28]. All samples were run in triplicate and the activity of lymphocyte AChE was expressed as µmol AcSCh/h/mg of protein.

2.6 Enzymatic assays in serum

ADA activity in serum was determined according to Guisti and Gakis, (1971) [29] as described above. BuChE activity was determined by a modification of the method by Ellman et al. (1961) [28]. This method is based on the formation of 5,5′-dithiobis(2-nitrobenzoic acid) measured at 412 nm. The reaction was initiated by adding butyrylthiocholine iodide (BuSCh). BuChE activity was expressed in µmol BuSCh/h/mg of protein.

2.7 Protein Determination

Protein was measured according to Bradford (1976) [30].

2.8 Statistical Analysis

Data were analyzed by analysis of variance (one-way ANOVA) followed by the Tukey multiple range test, and $P<0.05$ was considered to represent a significant difference in the analysis. All data were expressed as mean ± SEM.

3. Results

Table 1 showed that the consumption of HPD increased the levels of some metabolic parameters such as weight ($F(3, 23) = 11.86$, $P<0.001$), visceral fat mass ($F(3, 23) = 12.45$, $P<0.001$), blood glucose ($F(3,19) = 23.23$, $P<0.001$), total cholesterol ($F(3, 21) = 29.50$, $P<0.001$), cholesterol-LDL ($F(3,13) = 89.20$,

P<0.001), TAG (F(3,15) = 26.72, *P*<0.001), and IL-6 (F(3, 13) = 83.42, *P*<0.001) when compared to the control group. Treatment with both *P. cattleianum* and *E. uniflora* extracts prevented these changes caused by HPD.

HPD also altered the NTPDase, ADA, and AChE activities in the lymphocytes. Our results showed that HPD decreased ATP (F(3, 16) = 6.93, *P*<0.01; Fig. 2A) and ADP hydrolysis (F(3, 17) = 8.51, *P*<0.001; Fig. 2B), and treatments with extracts of *P. cattleianum* and *E. uniflora* only prevented the decrease of ATP hydrolysis. In addition, only *E. uniflora* treatment was able to prevent the increase in ADA activity in lymphocytes caused by HPD consumption (F(3, 17) = 18.50, *P*<0.001; Fig. 3A). Moreover, we observed that *P. cattleianum* and *E. uniflora* treatments also prevented the increase in AChE activity caused by HPD consumption (F(3, 12) = 7.70, *P*<0.01; Fig. 4A).

Similar results in relation to ectoenzyme activities were observed in the platelets. HPD also decreased ATP (F(3, 14) = 71.56, *P*<0.001; Fig. 5A) and ADP hydrolysis (F(3, 14) = 6.14, *P*<0.01; Fig. 5B) in the cells. Treatments with both fruit extracts were able to prevent these alterations in a similar manner. Furthermore, Fig. 5C shows that *P. cattleianum* and *E. uniflora* treatments prevented the decrease in the 5´-nucleotidase activity caused by consumption of HPD (F(3, 12) = 20.90, *P*<0.001). Conversely, no significant differences were observed in the activity of the ADA enzyme in the platelets from any of the groups tested (F(3, 12) = 3.25, *P*>0.05; Fig. 5D). In addition, we also evaluated the activity of enzymes ADA and BuChE in the serum of rats administered the HPD diet and treated with *P. cattleianum* and *E. uniflora* extracts. Our results show that both fruit extracts were able to prevent the increase of BuChE activity caused by HPD consumption (F(3, 20) = 6.75, *P*<0.01; Fig. 4B). Moreover,

consumption of HPD was able to increase the activity of the ADA enzyme; however, treatment with *P. cattleianum* and *E. uniflora* extracts did not prevent this increase in activity from occurring ($F(3, 14) = 5.98$, $P<0.01$; Fig. 3B).

4. Discussion

In the present study, we evaluated the effects of treatment with fruit extracts of *P. cattleianum* and *E. uniflora* on the changes in metabolic, inflammatory, and thromboregulatory parameters in rats with MetS induced by HPD consumption. Both extracts prevented the metabolic alterations induced by HPD, such as weight gain, visceral fat accumulation, hyperglycemia, and dyslipidemia (increase of total cholesterol, LDL-cholesterol, and TAG) as previously described by our research group [18, 19]. It is likely that the weight gain observed in animals receiving the HPD is related to increased visceral fat, given that visceral fat accumulation may be related to the development of other factors associated with MetS [18, 19, 21]. In addition, we observed that both extracts were able to prevent the increase in serum levels of IL-6 induced by the consumption of HPD. Likewise, Oliveira and collaborators (2018) [19] also showed that the red fruit extract of *P. cattleianum* was able to prevent the increase in serum levels of IL-6 in animals administered the HPD.

Previous studies have suggested that anthocyanins prevent the increase of visceral fat and improve the lipid and glucose profile and thus may contribute to a reduction in the production of pro-inflammatory cytokines, attenuating the inflammatory process in MetS [14, 31-33]. In fact, the inflammatory process seems to be a central mechanism in the pathophysiology of MetS, given that visceral adipose tissue may lead to increased production and secretion of

different pro-inflammatory cytokines, contributing to the development of chronic inflammation, of low grade, present in this syndrome [6, 11, 14].

The cholinergic pathway is also involved in the inflammatory process because it modulates immunity through the action of the ACh molecule in nicotinic receptors on the lymphocyte surface and therefore inhibits the production of pro-inflammatory cytokines [6, 9, 10, 34]. Considering this fact, we also analyzed the activity of AChE in lymphocytes and serum BuChE in the rat model of MetS. We observed that treatment with the extracts prevented the increase of the activities of these enzymes induced by the consumption of HPD. Increases in AChE and BuChE activity in patients with MetS have been reported previously [9, 11]. In addition, it has been suggested that the increase in BuChE activity may have a positive correlation with the development of cardiovascular diseases, diabetes, and MetS, because high serum lipid concentrations may induce stereoscopic alterations in an enzymatic configuration that modifies BuChE activity or alters the expression of the enzyme-encoding gene that regulates the concentration and activity of the enzymes [10, 11]. Moreover, when there is an increase in the activity of the cholinergic enzymes, degradation of ACh occurs; the reduction of this molecule may decrease its anti-inflammatory effect [9, 10]. Thus, a possible mechanism involved in the beneficial effects of *P. cattleianum* and *E. uniflora* extracts may be associated with the peripheral modulation of cholinergic signaling.

Considering that ectonucleotidases are important enzymes involved in the regulation of immune and inflammatory responses, we also evaluated the effect of *P. cattleianum* and *E. uniflora* extracts on the activity of the E-NTPDase and ADA enzymes in lymphocytes and serum of the rat model of MetS. Our results

showed that HPD decreased the E-NTPDase activity in lymphocytes and increased the ADA activity in lymphocytes and serum. On the other hand, treatment with the extracts played a modulatory role in the activity of these enzymes. This effect can be attributed to the anti-inflammatory property of anthocyanins, such as cyanidin 3-glycoside, present in the fruit extracts of *P. cattleianum* and *E. uniflora* [13, 14, 33]. In addition, Abdala and collaborators (2014) [35] demonstrated that flavonoids, such as quercetin, can inhibit ADA activity and consequently increase the concentration of adenosine, playing an important anti-inflammatory role and exhibiting immunosuppressive action. Moreover, adenosine can regulate several aspects of adipose tissue function including lipolysis, which is observed in MetS [9]. In fact, increased visceral fat in adipose tissue may contribute to the development of IR, chronic low-grade inflammation, oxidative stress, hyperglycemia, dyslipidemia, and endothelial dysfunction [3, 8, 9, 11, 36]. These effects, when associated, represent one of the main links between MetS and the increased risk of atherothrombotic and cardiovascular events [36]. The purinergic system also plays an important role in the control of thrombotic processes. In our study, we observed that HPD consumption caused a decrease in NTPDase and 5´-nucleotidase activity in platelets. However, treatment with *P. cattleianum* and *E. uniflora* prevented such changes from occurring. In agreement with our findings, Souza et al. (2010) [37] also observed a decrease in the E-NTPDase and 5´-nucleotidase activities in the serum of animals exposed to HPD by 120 days. The combined action of the ectonucleotidases converts ATP, ADP, and AMP to adenosine. Adenosine inhibits platelet aggregation and acts as a vasodilator; elevated levels of ATP and

ADP may induce platelet aggregation and is a potential risk factor for the development of cardiovascular diseases [8, 37, 38].

In MetS, chronic hyperglycemia and elevated glucose levels lead to an increase in extracellular ATP levels [7, 39]. The importance of extracellular ATP in the process of vasodilation and vasoconstriction, platelet aggregation, and inflammation is well established [3, 8, 37]. Acute release of ATP can activate nuclear factor kappa B that stimulates the encoding of several genes, including those responsible for producing pro-inflammatory cytokines such as IL-6, immune receptors, cell adhesion molecules, and acute-phase proteins [7]. Elevated cholesterol levels also may lead to platelet accumulation within atherosclerotic lesions, leading to the recruitment of additional platelets to form a thrombus, indicating that the arterial wall may assume an inflammatory and prothrombogenic phenotype [40]. The positive correlation between serum cholesterol and glucose concentration and ATP and ADP hydrolysis demonstrate that hyperglycemia and dyslipidemia are important factors capable of interfering with the activity of the ectonucleotidases [39, 40]. The modulating effect of the extracts tested on the activity of ectonucleotidases can be attributed, not only to their anti-inflammatory effect, but also to the ability of anthocyanins to improve the metabolism of glucose and lipids [13, 14, 33]. As already mentioned, the increase in serum glucose and cholesterol levels, when associated, are important risk factors that contribute to the imbalance of the thromboregulatory process. In addition, studies have shown that the consumption of red fruits rich in anthocyanins have been associated with a lower incidence of inflammatory, cardiovascular, and atherosclerotic coronary diseases, MetS, IR, and oxidative damage [13, 17-19, 21, 41].

Our findings indicate that MetS occurs with a combination of disorders associated with inflammatory changes and a prothrombotic condition characterized by an increase in IL-6 serum levels and changes in the activities of cholinesterase and ectonucleotidase enzymes. In addition, red fruit extracts of *P. cattleianum* and *E. uniflora* modulated the activities of these enzymes, contributing to anti-inflammatory and anti-thrombotic actions and improving the lipid and glucose profiles, which suggests cardiovascular protection. These extracts have demonstrated preventive effects against the complications associated with MetS and may be a potential therapeutic agent for the control and prevention of this syndrome.

Acknowledgments

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Conflict of interest

The authors declare that there are no conflicts of interest in this study.

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Figure captions

Figure 1. Experimental protocol.

Figure 2. Effects of treatment with *P. cattleianum* and *E. uniflora* fruit extracts on ATP (A) and ADP (B) hydrolysis in lymphocytes of a rat model of metabolic syndrome. Results are expressed as mean ± S.E.M. (n=5-6 for group). (**) *P*<0.01 when compared with vehicle/SC. (*) *P*<0.05 when compared with vehicle/SC. ($^{\#\#}$) *P*<0.01 when compared with vehicle/HPD. ($^{\#}$) *P*<0.05 when compared with vehicle/HPD. One-way ANOVA followed by Tukey *post-hoc* test. SC = Standard Chow; HPD= Highly Palatable Diet.

Figure 3. Effect of treatment with *P. cattleianum* and *E. uniflora* fruit extract son the adenosine deaminase (ADA) activity in lymphocytes (A) and serum (B) of a rat model of metabolic syndrome. Results are expressed as mean ± S.E.M. (n=5-6 for group). (***) *P*<0.001 when compared with vehicle/SC. ($^{\#\#}$) *P*<0.01 when compared with vehicle/HPD. One-way ANOVA followed by Tukey *post-hoc* test. SC = Standard Chow; HPD= Highly Palatable Diet.

Figure 4. Effect of treatment with *P. cattleianum* and *E. uniflora* fruit extracts on AChE activity in lymphocytes (A) and BuChE activity in serum (B) of a rat model of metabolic syndrome. Results are expressed as mean ± S.E.M. (n=5-6 for group). (**) *P*<0.01 when compared with vehicle/SC. (*) *P*<0.05 when compared with vehicle/SC. ($^{\#}$) *P*<0.05 when compared with vehicle/HPD. One-way ANOVA followed by Tukey *post-hoc* test. SC = Standard Chow; HPD= Highly Palatable Diet.

Figure 5. Effect of treatment with *P. cattleianum* and *E. uniflora* fruit extracts on ATP (A), ADP (B), AMP (C) and adenosine (ADO) (D) hydrolysis in platelets of a rat model of metabolic syndrome. Results are expressed as mean ± S.E.M. (n=5-6 for group). (**) $P<0.01$ when compared with vehicle/SC. (*) $P<0.05$ when compared with vehicle/SC. ($^{###}$) $P<0.001$ when compared with vehicle/HPD. ($^{##}$) $P<0.01$ when compared with vehicle/HPD. ($^{#}$) $P<0.05$ when compared with vehicle/HPD. One-way ANOVA followed by Tukey *post-hoc* test. SC = Standard Chow; HPD= Highly Palatable Diet.

Figure 1

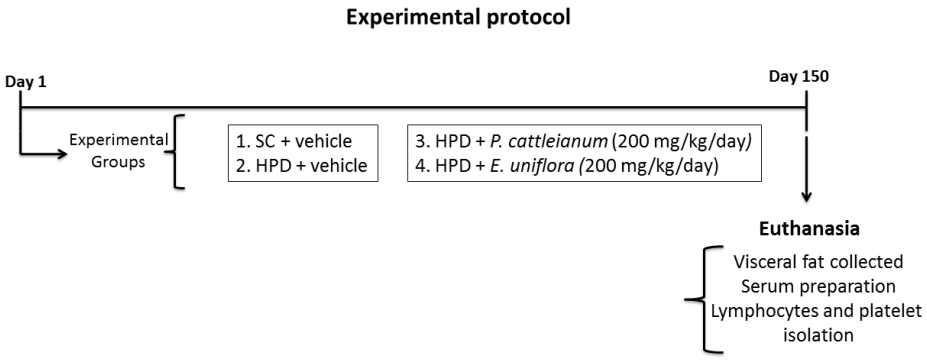
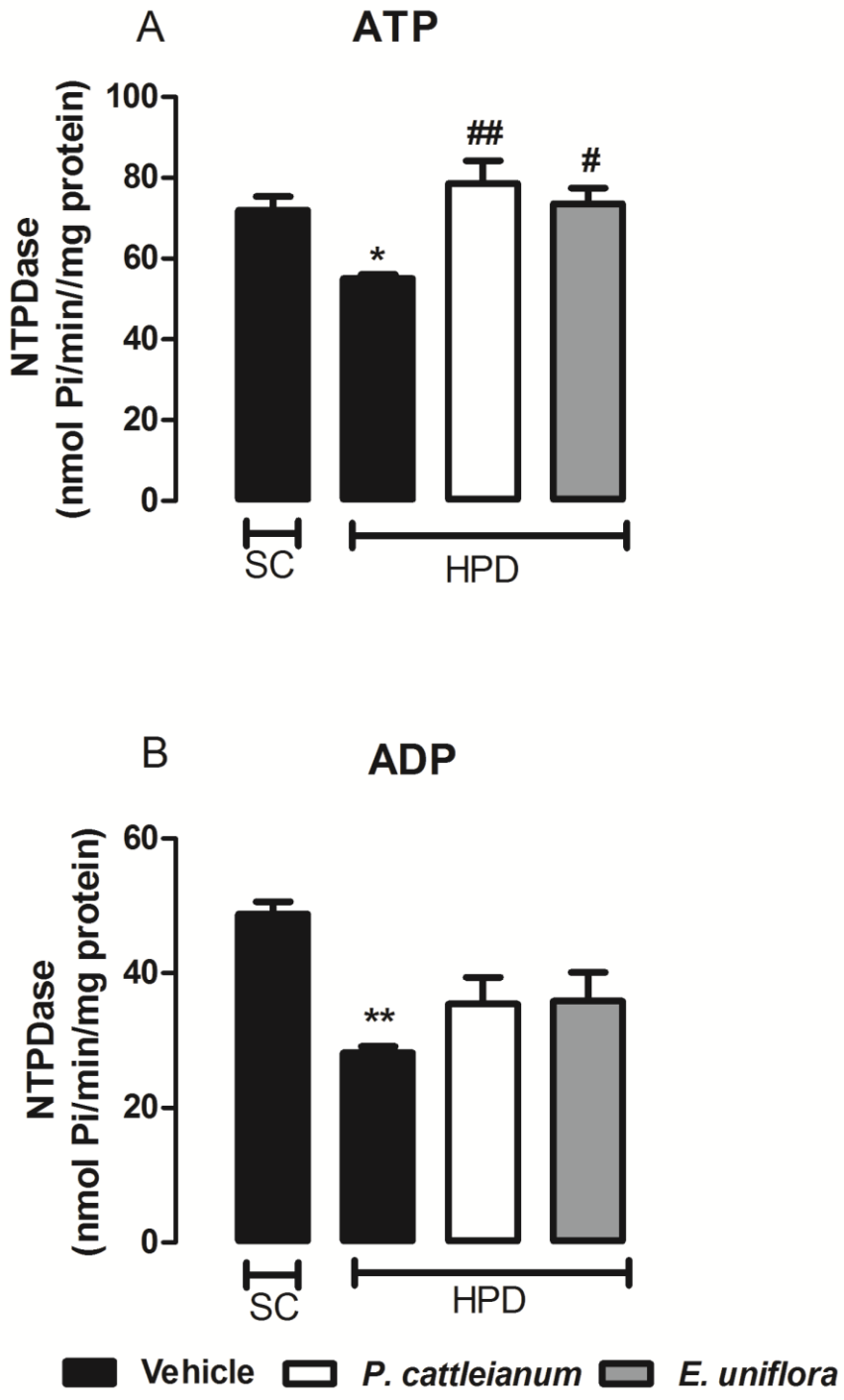

Experimental protocol
Day 1
Day 150
Experimental Groups
1. SC + vehicle
2. HPD + vehicle
3. HPD + P. cattleianum (200 mg/kg/day)
4. HPD + E. uniflora (200 mg/kg/day)
Euthanasia
Visceral fat collected
Serum preparation
Lymphocytes and platelet isolation

Figure 2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(nmol Pi/min//mg protein)
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SC
HPD
B
ADP
NTPDase
(nmol Pi/min/mg protein)
60
40
20
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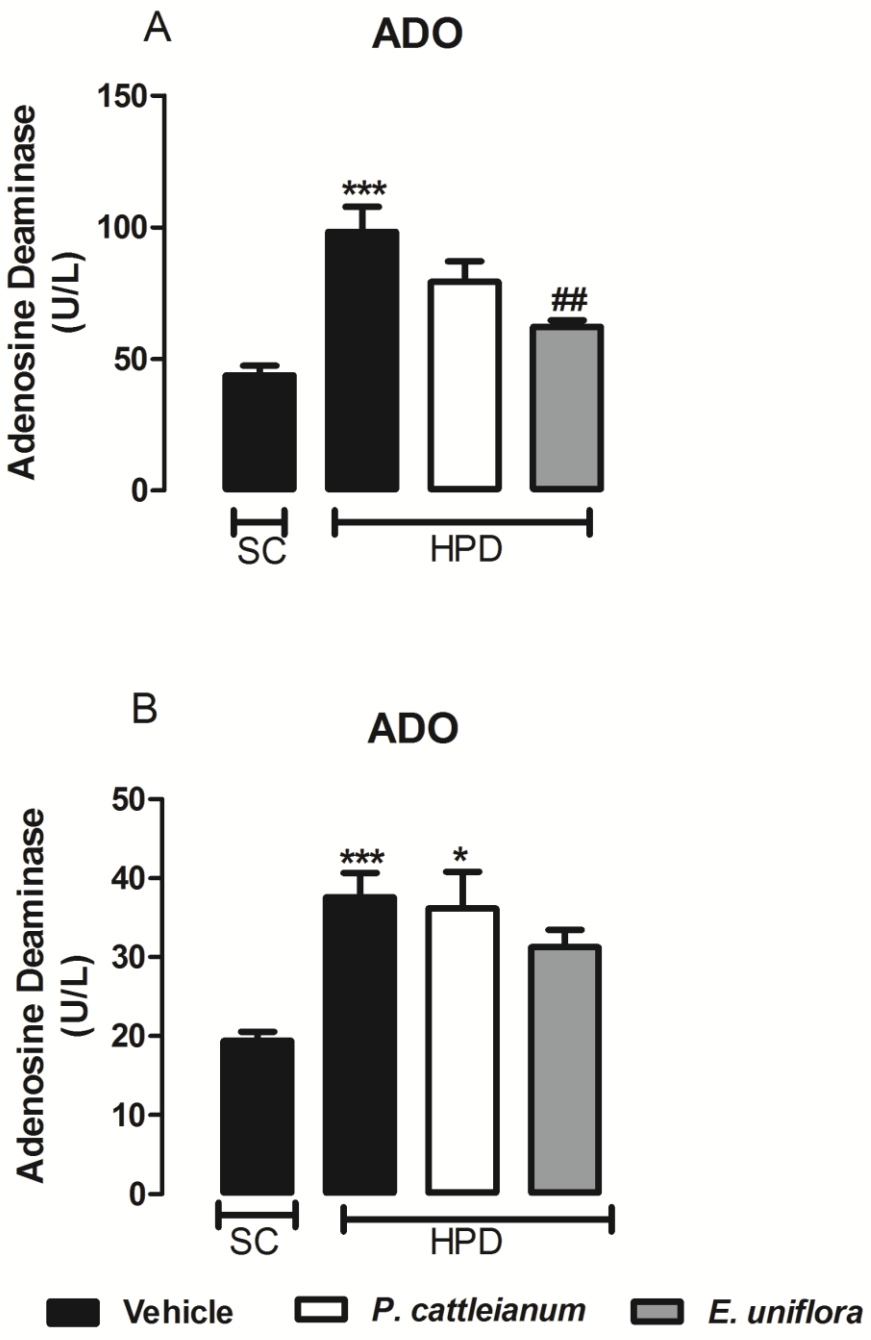
SC
HPD
Vehicle
P. cattleianum
E. uniflora

Figure 3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Adenosine Deaminase (U/L)
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SC
HPD
B
ADO
Adenosine Deaminase (U/L)
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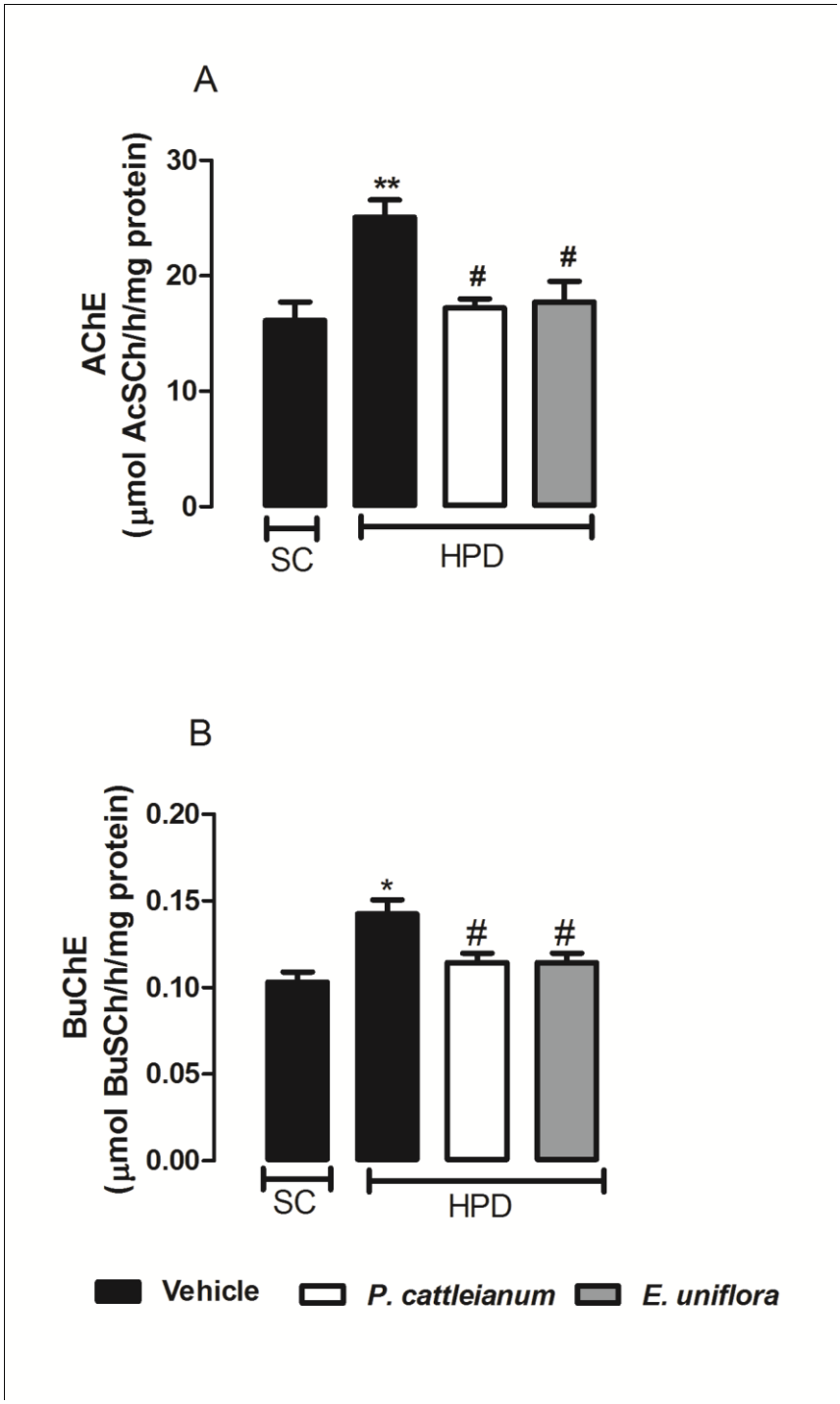
*
SC
HPD
Vehicle
P. cattleianum
E. uniflora

Figure 4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SC
HPD
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BuChE
(µmol BuSCh/h/mg protein)
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0.10
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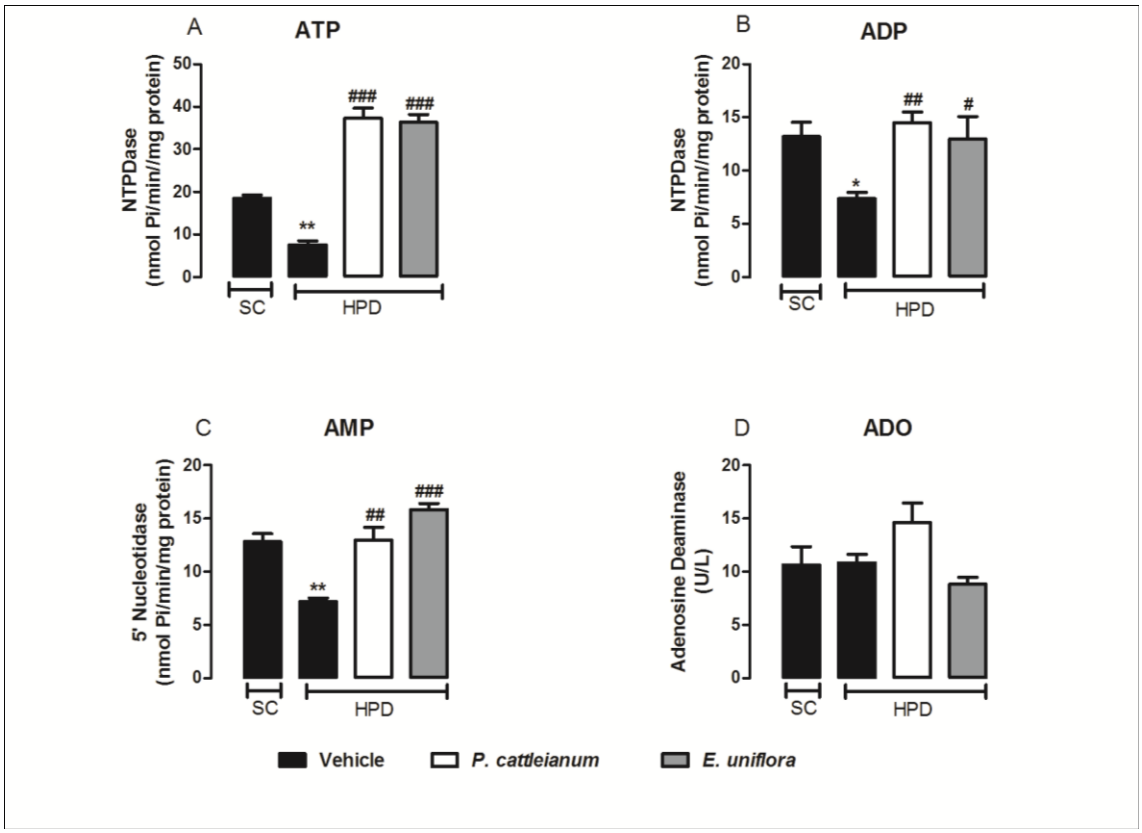
SC
HPD
Vehicle
P. cattleianum
E. uniflora

Figure 5


A
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SC
HPD
Vehicle
P. cattleianum
E. uniflora

Table 1. Effect of *P. cattleianum* and *E. uniflora* fruit extract treatment in rats exposed to a highly palatable diet on metabolic parameters

	SC/Vehicle	HPD/Vehicle	HPD/*P. cattleianum*	HPD/*E. uniflora*
Weight gain (g)	366±18.86	482±20.89***	386±6.13$^{###}$	404±7.73$^{##}$
Visceral fat mass (g)	16.89±1.62	40.56±5.07***	20.83±1.23$^{###}$	22.99±1.89$^{##}$
Glucose (mg/dL)	77.00±3.12	131.70±7.11***	102.70±1.93$^{##}$	89.42±4.69$^{###}$
Total cholesterol (mg/dL)	103.20±3.08	152.7±6.49***	104.20±0.90$^{###}$	109.60±2.09$^{###}$
LDL-cholesterol (mg/dL)	47.42±2.07	102.30±2.60***	35.77±3.74$^{###}$	45.55±3.95$^{###}$
Triacylglycerol (mg/dL)	66.11±7.91	98.91±2.59**	36.97±4.54$^{###}$	58.82±2.96$^{###}$
IL-6 (pg/mg protein)	0.73±0.02	1.38±0.04***	0.56±0.05$^{###}$	0.61±0.04$^{###}$

Data are expressed as mean ± S.E.M. (n =5-8). (***) P<0.001 as compared with SC/Vehicle. (*) P<0.05 as compared with SC/Vehicle. ($^{###}$) P<0.001 as compared with HPD/Vehicle. ($^{##}$) P<0.01 as compared with HPD/Vehicle. SC= Standard Chow; HPD= Highly Palatable Diet. One-way ANOVA followed by Tukey *post-hoc* test.

7. Considerações finais

No presente trabalho, investigamos a capacidade dos extratos de frutos vermelhos de *E. uniflora* e *P. catlleianum* nativos da região Sul do Brasil em prevenir alguns parâmetros metabólicos, inflamatórios, aterogênicos, neuroquímicos e comportamentais em animais submetidos ao modelo de SM induzida pelo consumo da DHP. Nossos resultados demonstraram que a DHP induziu a SM uma vez que alterou os parâmetros metabólicos analisados (ganho de peso, acúmulo de gordura visceral, intolerância a glicose, hiperglicemia e dislipidemia). No entanto, o tratamento com os extratos testados foi capaz de prevenir o desenvolvimento das alterações metabólicas induzidas pela DHP. O efeito protetor dos extratos testados pode ser atribuído à presença dos compostos fenólicos. Estudos têm demonstrado que esses compostos apresentam um importante efeito na sinalização da insulina, bem como na redução dos parâmetros metabólicos analisados (HANHINEVA et al., 2010; COLLINS et al., 2015; BHASWANT et al., 2015; VENDRAME et al., 2016). Além disso, esses compostos podem aumentar a oxidação de gorduras, bem como inibir enzimas digestivas, como α-amilase e a absorção de glicose no intestino (HANHINEVA et al., 2010; GUO et al., 2012; BHASWANT et al., 2015).

Neste estudo, observamos também que a DHP induziu o estresse oxidativo nas estruturas cerebrais testadas (hipocampo, estriado e córtex pré-frontal) uma vez que aumentou os níveis de TBARS, ERO e nitrito, bem como reduziu a atividade das enzimas antioxidantes SOD e CAT. Sabe-se, que o SNC é metabolicamente ativo e mais suscetível ao dano oxidativo (HALLIWELL & GUTTERIDGE, 2007). Além disso, o aumento energético pode levar ao aumento de ERO causando disfunção mitocondrial, aumentando a oxidação de glicose e levando a peroxidação lipídica (MADANI et al., 2015; TREVINO et al., 2015; JHA et al., 2017). Por outro lado, observamos que o tratamento com os extratos testados foi capaz de prevenir o desenvolvimento do estresse oxidativo observado nesse modelo. Este efeito protetor pode ser atribuído à ação antioxidante dos extratos testados. De fato, estudos têm demostrado que extratos de frutos vermelhos de *E. uniflora* e *P. catlleianum* exibem importantes ações antioxidantes (MEDINA et al., 2011; CARDOSO et al., 2018). Nesse sentido, avaliamos também a ação neuroprotetora desses extratos e

observamos que a DHP levou ao desenvolvimento de um comportamento tipo-depressivo observado através do aumento no tempo de imobilidade dos animais submetidos ao teste do nado forçado. Alguns estudos têm demonstrado que esse comportamento pode estar relacionado à alteração na atividade de enzimas importantes para neurotransmissão como Na^+,K^+-ATPase, Ca^{2+}-ATPase e AChE (GAMARO et al., 203; CARVALHO et al., 2015; QUINES et al., 2016). A alteração na atividade dessas enzimas também pode ser atribuída ao aumento da ingestão energética, bem como da hiperglicemia, os quais podem desencadear o estresse oxidativo e um estado pró-inflamatório, induzindo a oxidação de neurotransmissores, neuroinflamação e morte neural (TREVINO et al., 2015; CARVALHO et al., 2015). No entanto, os extratos de frutos vermelhos de *E. uniflora* e *P. catlleianum* foram capazes de prevenir as alterações neuroquímicas e comportamentais induzidas pela DHP demonstrando não apenas um efeito antioxidante, mas também neuroprotetor. Esse efeito pode ser atribuído aos polifenóis presentes nesses frutos (CARVALHO et al., 2015; BENSALEN et al., 2018; JOO et al., 2018).

A fim de melhor compreender a fisiopatologia da SM avaliamos alguns marcadores inflamatórios e tromboregulatórios em linfócitos, plaquetas e/ ou soro dos animais testados. Nesse sentido, observamos que a DHP propiciou um ambiente pró-inflamatório e pró-aterogênico uma vez que aumentou os níveis séricos de IL-6 e alterou a atividade de enzimas dos sistemas purinérgico e colinérgico, incluindo E-NTPDase, ADA, AChE e BuChE. No entanto, o tratamento com os extratos testados foi capaz de atenuar o processo inflamatório e aterogênico presente na SM. O efeito protetor dos extratos testados pode ser atribuído a seus efeitos anti-hiperglicêmicos e anti-hiperlipidêmicos, uma vez que tem sido relatado que a hiperglicemia pode levar ao aumento nos níveis de ATP extracelular (LUNKES et al., 2008). A liberação aguda de ATP para o meio extracelular pode estimular a codificação de vários genes, incluindo aqueles responsáveis pela produção de citocinas pró-inflamatórias (LUNKES et al., 2008). Além disso, a hipercolesterolemia pode levar ao recrutamento de plaquetas adicionais para formação de trombo indicando que a parede arterial pode desenvolver um fenótipo pró-inflamatório e pró-aterogênico, contribuindo para as alterações observados nos animais submetidos ao modelo de SM.

De modo geral, os extratos de *E. uniflora* e *P. cattleianum* demonstraram efeitos anti-hiperglicêmicos, anti-hiperlipidêmicos, anti-inflamatórios, antitrombóticos, bem como neuroprotetores em modelo animal de SM. Sendo assim, esses extratos podem ser considerados promissores agentes para o controle e prevenção dessa síndrome.

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Anexo

Carta de parecer do Cômite de Ética em Experimentação Animal

Comissão de Ética em Experimentação Animal UFPel

Pelotas, 22 de janeiro de 2014

De: Prof. Dr. Éverton Fagonde da Silva

Presidente da Comissão de Ética em Experimentação Animal (CEEA)

Para: Professora Francieli Moro Stefanello

Centro de Ciências Químicas, Farmacêuticas e de Alimentos

Senhora Professora:

A *CEEA* analisou o projeto intitulado: **"Avaliação farmacológica de frutos nativos em modelo animal de síndrome metabólica"**, processo nº23110.009125/2013-86, sendo de parecer **FAVORÁVEL** a sua execução, considerando ser o assunto pertinente e a metodologia compatível com os princípios éticos em experimentação animal e com os objetivos propostos.

Solicitamos, após tomar ciência do parecer, reenviar o processo à *CEEA*.

Salientamos também a necessidade deste projeto ser cadastrado junto ao Departamento de Pesquisa e Iniciação Científica para posterior registro no *COCEPE* (código para cadastro nº **CEEA 9125**).

Sendo o que tínhamos para o momento, subscrevemo-nos.

Atenciosamente,

Éverton Fagonde da Silva

Prof. Dr. Éverton Fagonde da Silva

Presidente da CEEA

Ciente em: 23/01/2014

Assinatura da Professora Responsável:

Francieli Moro Stefanello