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Centro de Ciências Químicas, Farmacêuticas e de Alimentos
Programa de Pós-Graduação em Bioquímica e Bioprospecção
Tese de Doutorado

Produtos naturais na prevenção de alterações bioquímicas, oxidativas e/ou histopatológicas associadas a doenças metabólicas: estudo em modelo animal de Hipotireoidismo e Diabetes *Mellitus* Tipo 2

Juliane de Souza Cardoso

Pelotas, 2022

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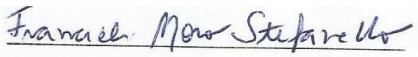

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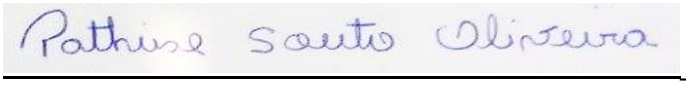

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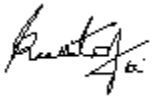

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RESUMO

CARDOSO, Juliane de Souza. **Produtos naturais na prevenção de alterações bioquímicas, oxidativas e/ou histopatológicas associadas a doenças metabólicas: estudo em modelo animal de Hipotireoidismo e Diabetes *Mellitus* Tipo 2.** 2022. 96f. Tese de doutorado - Programa de Pós-Graduação em Bioquímica e Bioprospecção, Universidade Federal de Pelotas, Pelotas, 2022.

Hipotireoidismo e Diabetes *Mellitus* tipo 2 (DM2) são duas importantes doenças que afetam significativamente a qualidade de vida dos pacientes e aumentam o risco de desenvolvimento de outras patologias. Dentre os mecanismos envolvidos na fisiopatologia e no agravo do hipotireoidismo e da DM2 estão principalmente o estresse oxidativo e o déficit colinérgico. Tendo em vista a complexidade dessas doenças, a forte relação dessas com desordens cognitivas, bem como a ineficácia dos tratamentos existentes na prevenção ou reversão das alterações encontradas nos pacientes portadores, torna-se necessária a busca por novas alternativas terapêuticas. Diante disso, este trabalho se propôs a avaliar os possíveis efeitos preventivos do resveratrol e do *Psidium cattleianum* (*P. cattleianum*) frente as alterações metabólicas, bioquímicas, oxidativas, colinérgicas e histológicas decorrentes do hipotireoidismo e da DM2. Para isso, foram utilizados ratos Wistar adultos machos em modelos experimentais de hipotireoidismo, induzido por metimazol, e de DM2, induzido por dieta hiperlipídica e dose única de estreptozotocina. Em ambos os modelos estudados, durante o período experimental de 30 dias, os animais receberam diariamente, uma vez ao dia, resveratrol ou extrato hidroalcoólico de *P. cattleianum* pela via intragástrica. Os resultados referentes ao modelo de hipotireoidismo mostraram que o resveratrol, apesar de não promover o aumento dos níveis de hormônios tireoidianos, preveniu significativamente o aumento da atividade da enzima acetilcolinesterase, prevenindo o déficit colinérgico. Além disso, preveniu o aumento dos níveis de espécies reativas de oxigênio (ERO) em soro e cérebro, e o aumento dos níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS), e a diminuição da atividade da enzima catalase (CAT) e do conteúdo tiólico total em cérebro de animais hipotireoidianos. Sendo assim, o resveratrol mostrou-se um potencial agente neuroprotetor e antioxidante. Em modelo de DM2, o extrato de *P. cattleianum* preveniu alterações bioquímicas séricas, como aumento de glicose, colesterol total, triacilglicerois e VLDL, além de prevenir o aumento dos níveis de ERO, TBARS e a diminuição da atividade da enzima CAT e superóxido dismutase. Além disso, protegeu o fígado e pâncreas de alterações celulares, estruturais e necrose decorrentes da DM2 induzida. Dessa forma, o extrato de *P. cattleianum* mostrou-se um promissor antioxidante, anti-hiperglicêmico e antilipêmico. Diante dos resultados apresentados, foi possível compreender o papel dos produtos naturais aqui estudados em modelos animais de hipotireoidismo e DM2 e considerá-los eficazes na prevenção dessas doenças metabólicas e de seus agravos.

Palavras-chave: doenças metabólicas; sistema colinérgico; *status* redox; resveratrol; frutos nativos

ABSTRACT

CARDOSO, Juliane de Souza. **Natural products in the prevention of biochemical, oxidative and/or histopathological alterations associated with metabolic diseases: study in an animal model of Hypothyroidism and Type 2 Diabetes *Mellitus*.** 2022. 96f. Doctoral Thesis - Graduate Program in Biochemistry and Bioprospecting, Federal University of Pelotas, Pelotas, 2022.

Hypothyroidism and Type 2 Diabetes Mellitus (DM2) are two important diseases that significantly affect the quality of life of patients and increase the risk of developing other pathologies. Among the mechanisms involved in the pathophysiology and aggravation of hypothyroidism and T2DM are mainly oxidative stress and cholinergic deficit. Given the complexity of hypothyroidism and DM2, the strong relationship between these and cognitive disorders, as well as the ineffectiveness of existing treatments in preventing or reversing the changes found in patients with these disorders, it is necessary to search for new therapeutic alternatives. Therefore, this study aimed to evaluate the possible preventive effects of resveratrol and *Psidium cattleianum* (*P. cattleianum*) against metabolic, biochemical, oxidative, cholinergic and histological changes resulting from hypothyroidism and DM2. For this, adult male Wistar rats were used in experimental models of hypothyroidism, induced by methimazole, and of DM2, induced by a high-fat diet and a single dose of streptozotocin. In both models studied, during the experimental period of 30 days, the animals received daily, once a day, resveratrol or hydroalcoholic extract of *P. cattleianum* by the intragastric route. The results referring to the hypothyroidism model showed that resveratrol, despite not promoting an increase in the levels of thyroid hormones, significantly prevented the increase in the activity of the enzyme acetylcholinesterase, preventing the cholinergic deficit. Furthermore, it prevented the increase in the levels of reactive oxygen species (ROS) in serum and brain, and the increase in the levels of thiobarbituric acid reactive substances (TBARS), and the decrease in catalase enzyme (CAT) activity and total thiol in the brain of hypothyroid animals. Thus, resveratrol proved to be a potential neuroprotective and antioxidant agent. In a T2DM model, the *P. cattleianum* extract prevented serum biochemical alterations, such as an increase in glucose, total cholesterol, triacylglycerols and VLDL, in addition to preventing an increase in ROS and TBARS levels and a decrease in CAT and superoxide dismutase enzyme activity. In addition, it protected the liver and pancreas from cellular and structural changes and necrosis resulting from induced T2DM. Thus, the *P. cattleianum* extract proved to be a promising antioxidant, antihyperglycemic and antilipemic. In view of the results presented, it was possible to understand the role of natural products studied here in animal models of hypothyroidism and T2DM and to consider them effective in preventing these metabolic diseases and their aggravations.

Keywords: metabolic diseases; cholinergic system; redox status; resveratrol; native fruits

Lista de abreviaturas, siglas e símbolos

ACh- Acetilcolina

AChE- Acetilcolinesterase

AGE- Produtos finais de glicação avançada

AGL- Ácidos graxos livres

AKT ou PKB- Proteína cinase B

ATP- Adenosina trifosfato

BuChE- Butirilcolinesterase

cAMP- Adenosina 3',5'-monofosfato cíclico

CAT- Catalase

ChAT- Colina acetiltransferase

DA- Doença de Alzheimer

DAG- Diacilglicerol

DCNT- Doenças crônicas não transmissíveis

DCV- Doenças cardiovasculares

DIO- Iodotironina desiodinases

DM- Diabetes mellitus

DM1- Diabetes mellitus tipo 1

DM2- Diabetes mellitus tipo 2

DNA- Ácido desoxidoribonucleico

DPP-4- Dipeptidil peptidase 4

ERO- Espécies reativas de oxigênio

GAD65- Ácido glutâmico descarboxilase

GFAP- Proteína ácida fibrilar glial

GIP-1- Polipeptídeo inibidor gástrico 1

GLP-1- Peptídeo semelhante ao glucagon 1

GLP-1RA- Agonistas do receptor de peptídeo semelhante ao glucagon 1

GLUT- Transportador de glicose

GLUT4- Transportador de glicose 4

GPx- Glutationa peroxidase

GST- Glutationa peroxidase

H_2O_2- Peróxido de hidrogênio

HbA1c- Hemoglobina glicada

HC- Hipotireoidismo congênito

HDL- Lipoproteína de alta densidade

HT- Hormônios tireoidianos

IA-2- Antígeno de ilhota-2

IL-10- Interleucina 10

IL1-β- Interleucina 1β

IL-6- Interleucina 6

InR- Receptor de insulina

IRS- Substrato do receptor de insulina

LDL- Lipoproteína de baixa densidade

mAChRs- Receptores muscarínicos

MDA- Malondialdeído

nAChRs- Receptores nicotínicos

NADPH- Nicotinamida adenina dinucleotideo fosfato

NeuN- Marcador nuclear neuronal

NO- Óxido nítrico

Nrf2- Fator nuclear derivado do eritroide-2

O_2- Oxigênio

$O_2^{\bullet -}$- Ânion superóxido

$OH^{\bullet}$- Radical hidroxila

$ONOO^-$- Peroxinitrito

PI3K- Fosfoinositídeo-3-cinase

PIP2- Fosfatidilinositol-4,5-bisfosfato

PIP3- Fosfatidilinositol-3,4,5-trisfosfato

RI- Receptor de insulina

RI- Resistência à insulina

Ser- Serina

SGLT- Inibidores do cotransportador sódio-glicose

SM- Síndrome metabólica

SNC- Sistema nervoso central

SNP- Sistema nervoso periférico

SOD- Superóxido dismutase

STZ- Estreptozotocina

T2DM- *Type 2 diabetes mellitus*

T3- Tri-iodotironina

T4- Tetraiodotironina ou Tiroxina

TA- Tecido adiposo

TBARS- Substâncias reativas ao ácido tiobarbitúrico

TG- Triglicerídeo

TNF-α- Fator de necrose tumoral α

TOTG- Teste oral de tolerância à glicose

TPO- Tireoperoxidase

TR- Receptor de hormônio tireoidiano

TRH- Tireotrofina

TSH- Hormônio estimulante da tireoide

VAChT- Transportador vesicular de ACh

VLDL- Lipoproteína de muito baixa densidade

Lista de Figuras

Sumário

1 INTRODUÇÃO

O hipotireoidismo é uma doença caracterizada por baixos níveis de hormônios tireoidianos (HT), que acomete em torno de 10% da população, e vem sendo associado ao maior risco de desenvolvimento de resistência à insulina (RI), dislipidemia, hipertensão arterial, doenças cardiovasculares (DCV) e mortalidade (BIONDI; CAPPOLA; COOPER, 2019). A Diabetes *Mellitus* tipo 2 (DM2) é uma doença crônica grave, caracterizada por ineficiente ação do hormônio insulina, resultando em hiperglicemia. Diversas outras complicações decorrem da DM2, como insuficiência renal, cegueira, amputação de membros inferiores, alterações no sistema nervoso, dentre outras, que comprometem a qualidade de vida dos indivíduos e aumentam consideravelmente o risco de morte (WHO, 2016).

Estudos demonstram que indivíduos acometidos por hipotireoidismo e DM2 comumente apresentam transtornos de humor e problemas cognitivos, como déficit de atenção, memória e aprendizagem, e dentre os mecanismos envolvidos destacam-se o estresse oxidativo e o déficit colinérgico (DOMINGUES et al., 2018; NJAN et al., 2020). O estresse oxidativo é um quadro caracterizado por acúmulo de espécies reativas de oxigênio (ERO), que alteram diversas vias de sinalização celular, favorecendo muitos processos patológicos, dentre eles inflamação, diabetes, aterosclerose e neurodegeneração (ZHANG et al., 2016). O déficit colinérgico, por sua vez, é um comprometimento na via de sinalização colinérgica, a qual é responsável pelas funções cognitivas, como aprendizado, memória, atenção e emoção. Assim, o déficit colinérgico está intimamente envolvido nas alterações cognitivas observadas em portadores de doenças metabólicas (FERREIRA-VIEIRA et al., 2016).

O tratamento do hipotireoidismo é feito com reposição hormonal oral, visando manter níveis adequados dos HT. No entanto, o tempo de início do tratamento e a dose a ser utilizada ainda é motivo de discussão, dada a complexidade da fisiopatologia e diferentes tipos de hipotireoidismo (MATEO & HENNESSEI, 2019). Já o tratamento da DM2 baseia-se principalmente na adoção de um estilo de vida saudável, mas também conta com uma variedade de medicamentos que visam a normalização da glicemia. No entanto, nem sempre os medicamentos são eficazes e, ainda, podem ocasionar importantes efeitos adversos (KANECO & NARUKAVA, 2016).

Diante disso, torna-se necessária a busca de novas alternativas terapêuticas para o hipotireoidismo e a DM2. Nesse contexto, produtos naturais, como compostos fenólicos isolados ou frutos ricos em compostos fenólicos, parecem alternativas relevantes. Os compostos fenólicos compreendem uma ampla variedade de substâncias bioativas, com importantes atividades benéficas à saúde já comprovadas, destacando-se as atividades antioxidante e neuroprotetora (PENUMALA et al., 2018). O resveratrol é um polifenol do grupo dos estilbenos, que já demonstrou melhorar alguns déficits cognitivos, possivelmente por suas atividades antioxidante e anti-inflamatória (GE et al., 2015, 2016; TIAN et al., 2016). O fruto de *Psidium cattleianum* (*P. cattleianum*), conhecido popularmente como “araçá”, é um fruto brasileiro da família Myrtaceae, amplamente cultivado no Sul do país, rico em compostos fenólicos, que confere a ele importante atividade antioxidante e neuroprotetora já demonstradas pelo nosso grupo de pesquisa (CARDOSO et al., 2018; OLIVEIRA et al., 2018, 2020).

Assim, diante das atividades promissoras para promoção e recuperação da saúde obtidas pelo uso de produtos naturais, esta tese propõe-se a investigar os efeitos do composto isolado resveratrol em modelo experimental de hipotireoidsimo e de extrato padronizado de *P. cattleianum* em modelo experimental de DM2, através da avaliação dos efeitos desses em parâmetros metabólicos, bioquímicos séricos, oxidativos, colinérgicos e histológicos.

2 OBJETIVOS

2.1 Objetivo geral

Investigar os efeitos do composto isolado resveratrol em modelo experimental de hipotireoidsimo e de extrato padronizado de *P. cattleianum* em modelo experimental de DM2, em relação a parâmetros metabólicos, bioquímicos, oxidativos, colinérgicos e histológicos.

2.2 Objetivos específicos

a) Em modelo animal de hipotireoidismo induzido por metimazol, avaliar o efeito protetor do resveratrol em relação aos seguintes parâmetros:

- Bioquímicos séricos:
    - Níveis de T3 e T4;

- Estresse oxidativo em SNC e soro:
    - Níveis de ERO, nitrito, conteúdo tiólico total e TBARS;
    - Atividade das enzimas antioxidantes CAT e SOD;
- Neuroquímicos:
    - Atividade e expressão gênica da enzima AChE;
    - Densidade dos receptores colinérgicos α7 e M1;
    - Marcador nuclear neuronal (NeuN) e proteína glial fibrilar ácida (GFAP) em estruturas cerebrais.

b) Em modelo animal de DM2 induzida por dieta hiperlipídica e STZ, avaliar o efeito de extratos hidroalcoólicos dos frutos nativos vermelhos *P. cattleianum* em relação aos seguintes parâmetros:

- Metabólicos:
    - Ganho de peso total e peso final;
    - Peso relativo do pâncreas e do tecido adiposo (TA) visceral;

- Bioquímicos séricos:
    - TOTG, para avaliar a concentração de glicose no sangue, após sobrecarga oral de glicose;
    - Glicemia e perfil lipídico;
    - Quantificação da citocina pró-inflamatória IL-6 e anti-inflamatória IL-10;
    - Atividade da enzima paraoxonase-1;

- Estresse oxidativo em SNC:
    - Níveis de ERO, nitrito, conteúdo tiólico total e TBARS;
    - Atividade das enzimas antioxidantes CAT e SOD;

- Análise histológica de fígado e pâncreas.

3 REFERENCIAL TEÓRICO

3.1 Hipotireoidismo

O hipotireoidismo é uma doença caracterizada por níveis diminuídos de HT circulantes e/ou insuficiente resposta do organismo a esses hormônios, que podem resultar em fraqueza, distúrbios emocionais, DCV, infertilidade, malformação cerebral fetal e mortalidade (BIONDI; CAPPOLA; COOPER, 2019). A prevalência na Europa, na população em geral, é de aproximadamente 5,3% (CHIOVATO; MAGRI; CARLÉ, 2019), dentre os americanos 5 a cada 100 americanos com 12 anos ou mais possuem hipotireoidismo, com maior prevalência entre mulheres e pessoas com mais idade (PATIL; REHMAN; JIALAL, 2022). No Brasil, dados apontam uma prevalência de 12,3% em mulheres adultas e 19,1% em mulheres idosas no Rio de Janeiro, e em São Paulo a prevalência encontrada foi de 11,1% e 8,7% em mulheres e homens, respectivamente (BENSENOR, 2019). A relação do hipotireoidismo com a idade vem sendo explicada por alguns mecanismos, dentre eles a diminuição na secreção hipotalâmica de hormônio liberador de tireotrofina (TRH), redução da resposta da glândula tireoide ao hormônio estimulante da tireoide (TSH), menor transformação de tiroxina (T4) à tri-iodotironina (T3) e redução do transporte dos HT para os tecidos (MOORADIAN, 2019).

Os HT auxiliam no funcionamento de diversos órgãos, como rim, fígado e coração e, principalmente atuam no metabolismo energético através do aumento da termogênese, melhora do perfil lipídico, redução do acúmulo de gordura e aumento da degradação de gordura (LOUZADA & CARVALHO, 2018). Além disso, esses hormônios são essenciais para a formação e diferenciação neural normais (BRENT, 2012). A síntese dos HT ocorre no folículo tireoidiano e conta com a ação da enzima tireoperoxidase (TPO), da presença de iodo e da glicoproteína tireoglobulina (SILVERTHORN, 2017). A secreção desses hormônios ocorre a partir da interação hipotálamo-hipófise-tireoide, envolvendo a ação do TRH e do TSH. O TRH secretado pelo hipotálamo controla a secreção de TSH pela adenohipófise que, por sua vez, atua na glândula tireoide estimulando a síntese dos HT (SILVERTHORN, 2017) (Figura 1). Sabe-se que a maior parte dos hormônios secretados pela tireoide é T4, e que T3, a forma biologicamente ativa dos HT, tem 80% da sua quantidade diária necessária formada a partir de T4 pelas enzimas pertencentes à família das iodotironina desiodinases (DIO) (LUONGO; DENTICE; SALVATORE, 2019).

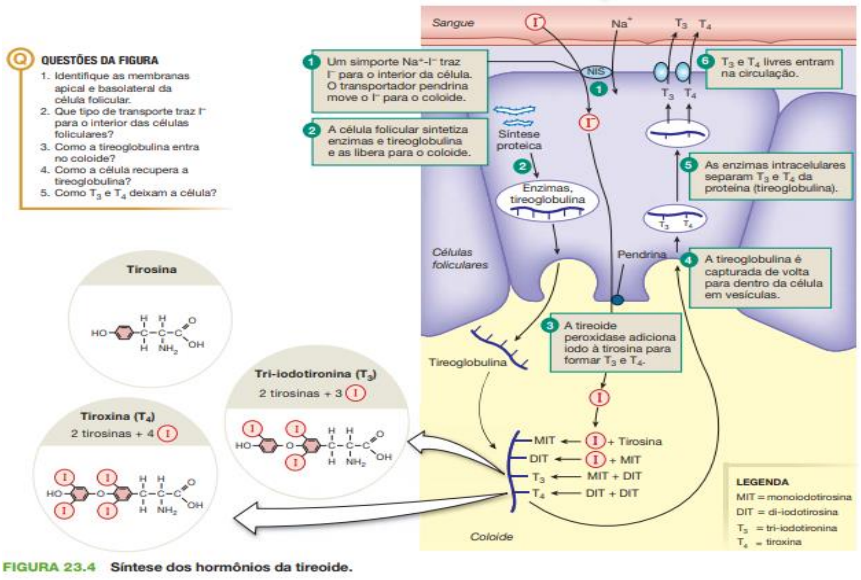

FIGURA 23.4 **Síntese dos hormônios da tireoide.**

Figura 1: Ilustração da síntese e secreção dos hormônios tireoidianos. Fonte: SILVERTHORN, 2017.

O hipotireoidismo é classificado, de acordo com sua etiologia, em primário, secundário, terciário e congênito. No primário, há uma falha da glândula em produzir T3 e T4, frequentemente causada por uma deficiência de iodo (HOERMANN et al., 2019). Dentro dessa classificação ainda há o hipotireoidismo autoimune, conhecido como doença de Hashimoto, onde há infiltração linfocitária da tireoide por autoanticorpos, principalmente antitireoide peroxidase (antiTPO) e antitireoglobulina (antiTG), que ao atacar e destruir esses componentes importantes da glândula inibem a síntese dos HT (LIONTIRIS & MAZOKOPAKIS, 2017). O hipotireoidismo secundário é decorrente de uma falência hipofisária e consequente falta de ação do TSH em estimular a produção dos HT. No hipotireoidismo terciário, apesar da produção normal de T4 pela glândula, há uma capacidade de resposta reduzida dos órgãos-alvo, ou seja, resistência aos HT ou, ainda, uma falha na conversão de T4 em T3 (GHANBARI & GHASEMI, 2017). O hipotireoidismo congênito (HC) é quando a criança nasce com deficiência de HT, podendo esse quadro ser transitório ou permanente, ocorre principalmente por anomalias na formação da glândula tireoide ou, ainda, na síntese, metabolismo e transporte dos HT. O HC transitório geralmente é atribuido a distúrbios maternos da tireoide relacionados à deficiência de iodo, ocorrendo mais em países deficientes desse mineral (GHANBARI & GHASEMI, 2017).

O diagnóstico do hipotireoidismo é realizado por quantificação dos níveis séricos de T4 e/ou TSH, podendo, a partir desses parâmetros bioquímicos, ser classificado ainda como manifesto (ou clínico) ou subclínico. No hipotireoidismo manifesto o TSH, que possui como valores de referência 0,4-4,0 mU/L, encontra-se persistentemente elevado em associação a níveis de T4 baixos. No hipotireoidismo subclínico, apesar de níveis normais de T4, níveis de TSH encontram-se elevados, classificando-o como leve (TSH: 4,5-9,9 mU/L) ou grave (TSH: ≥10 mU/L) (CHAKER et al., 2017).

O tratamento do hipotireoidismo baseia-se na terapia de reposição hormonal, que visa normalizar os níveis de HT e TSH e eliminar os sinais e sintomas da deficiência, como fadiga, ganho de peso, cabelos e pele secos, constipação, dislipidemia, hipertensão, disfunção endotelial, RI, dentre outros (BIONDI & COOPER, 2019). A reposição ocorre de forma padrão, a partir do hormônio sintético L-tiroxina, na dose usual diária de 1,5-1,8 µg/kg de peso corporal de pessoas jovens sem demais comorbidades. Já em mulheres gestantes a dose diária deve ser aumentada em 30% visando evitar consequências para a gestante e para o feto. Em pacientes com desordens coronárias, idosos e/ou com outras comorbidades, a introdução do hormônio deve ser feita de forma gradativa (CHAKER et al., 2017).

3.2 Diabetes *Mellitus* tipo 2

Diabetes *Mellitus* (DM) é um importante problema de saúde pública, enquadrada entre as quatro principais doenças crônicas não transmissíveis (DCNT), decorrente da ausência ou insuficiência na produção de insulina, ou ainda, deficiência em sua ação (WHO, 2019). Atualmente, há cerca de 463 milhões de adultos com DM, com idade entre 20 e 79 anos, o que representa 9,3% da população mundial pertencente à essa faixa etária. A estimativa é de que a prevalência aumente para 578 milhões em 2030 e até 700 milhões em 2045. Importante destacar que essa doença gera um custo mundial em saúde de, em média, 700 bilhões de dólares anualmente (IDF, 2019). Ainda, a hiperglicemia como fator isolado é responsável por cerca de 3,7 milhões de mortes por ano, sendo 1,5 milhões de pacientes diagnosticados com DM e 2,2 milhões de mortes por DCV e outras decorrentes da hiperglicemia (WHO, 2016).

A insulina é o hormônio secretado pelas células β das ilhotas pancreáticas de Langerhans em resposta ao aumento dos níveis circulantes de glicose, sendo responsável pela captação da glicose pelas células e, pelos diversos processos metabólicos e manutenção da glicemia (WHO, 2019). Ao ser liberada, na presença de glicose no sangue, a insulina diminui a gliconeogênese e a glicogenólise e aumenta a captação de glicose pelos tecidos periféricos, principalmente músculo e TA. Além disso, a insulina regula o metabolismo de lipídeos e proteínas, estimulando a lipogênese e reduzindo a lipólise e, ainda, aumentando a síntese e inibindo a degradação proteica (CARVALHEIRA et al., 2012; XU et al., 2014).

A via de sinalização da insulina compreende as reações que ocorrem desde a ligação do hormônio insulina nos receptores membranais até a captação da glicose para o interior da célula e, compreende várias etapas, conforme descrito a seguir: primeiramente, ocorre a ligação da insulina na subunidade α (extracelular) do receptor de insulina (InR), que a partir de uma mudança conformacional se auto-fosforila na sua subunidade intracelular β (intracelular) (DIONÍSIO et al., 2014). Logo, utilizando moléculas de adenosina trifosfato (ATP) a subunidade β do InR transmite o sinal para os substratos do receptor de insulina (IRS), que incluem IRS-1, IRS-2, IRS-3, IRS-4, Shc, Gab-1, p60dok, Cbl, JAK2 e APS, forforilando-os em resíduos de tirosina (Zhang & Liu, 2014). A ativação de IRS-1/2 leva à ativação da fosfoinositídeo-3-cinase (PI3K), que por sua vez catalisa a fosforilação da fosfatidilinositol-4,5-bisfosfato (PIP2) à fosfatidilinositol-3,4,5-trisfosfato (PIP3), que são constituintes lipídicos daS membranas e que em níveis aumentados levam ao recrutamento da proteína-quinase B (PKB ou AKT) do citosol para a superfície celular e à sua ativação (XU et al., 2014). A entrada da glicose para o interior da célula ocorre por difusão facilitada, considerando o gradiente de concentração, passando pelos transportadores de glicose (GLUT) através da membrana plasmática celular (SALTIEL, 2021) (Figura 2).

Foram identificadas 14 isoformas de transportadores, distribuídas em diferentes tecidos, porém apenas o GLUT-4 é dependente da sinalização da insulina e é altamente expresso nos tecidos muscular (cardíaco e esquelético) e TA (branco e marrom) (KRAFT et al., 2015). O último passo da via de sinalização da insulina, chamado translocação do GLUT4, ainda não é bem elucidado, porém evidências consideráveis apontam para a relação da AKT e seu substrato, a proteína AS160,

com a exocitose de vesículas especializadas contendo GLUT4 para a superfície celular (ZEIGERER et al., 2004).

O IRS-1 é altamente expresso no músculo esquelético, enquanto o IRS-2 encontra-se principalmente no tecido hepático e pancreático, onde desempenham importante papel funcional e, ambos estão associados a estruturas intracelulares (MOURÃO, 2020). O IRS-3 é expresso em adipócitos e a expressão do IRS-4 é praticamente restrita à glândula pituitária e ao cérebro, estando os dois associados à membrana plasmática (MOURÃO, 2020). Animais sem os genes que codificam IRS-1 apresentam RI e retardo de crescimento, sem apresentar hiperglicemia, enquanto animais sem o gene codificador de IRS-2 apresentam hiperglicemia acentuada, falência das células β-pancreáticas e redução da massa pancreática. Por outro lado, os animais deficientes de expressão de IRS-3 e IRS-4 apresentam crescimento e metabolismo quase normal (SALTIEL, 2021).

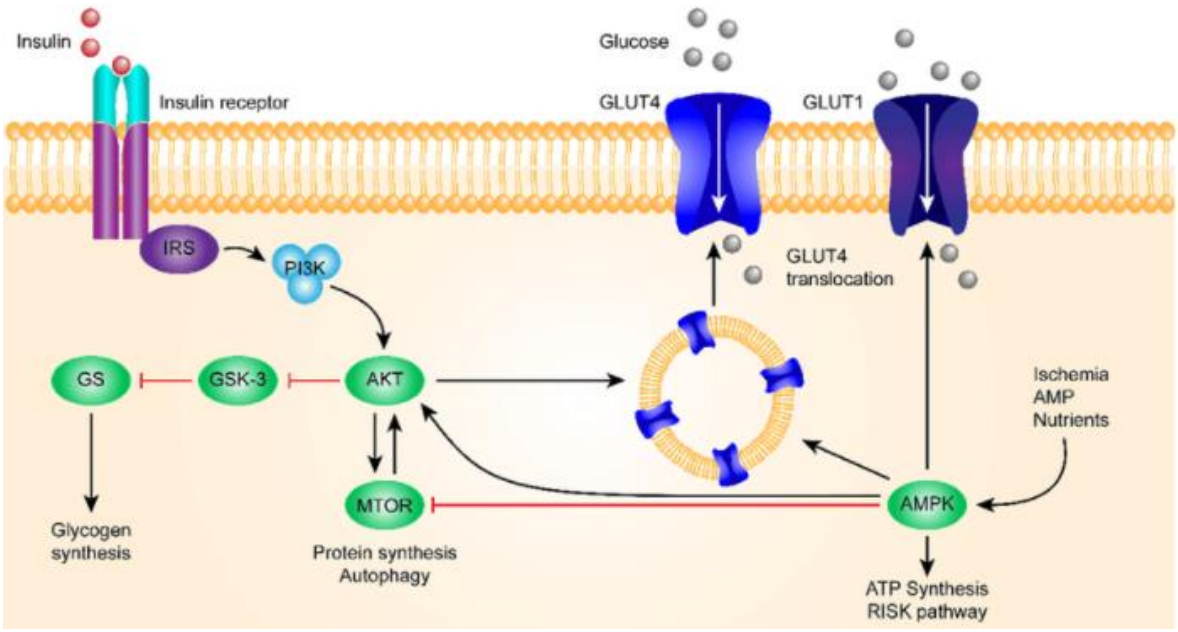

Figura 2: Via de sinalização da insulina iniciada pela ligação da insulina no receptor até a translocação do transportador de glicose (Fonte: Arneth et al., 2019).

Os sintomas clássicos da DM são decorrentes da hiperglicemia e incluem poliúria, polidipsia, polifagia e perda involuntária de peso e, além destes, fadiga, fraqueza, letargia, prurido cutâneo e vulvar e infecções de repetição devem ser considerados (DSBD,2019). Diversas outras complicações decorrem da DM, como retinopatia, nefropatia, neuropatia, DCV, doenças infecciosas, amputação de

membros inferiores e outras, que aumentam o risco de morte e afetam negativamente a qualidade de vida dos indivíduos (WHO, 2016).

A classificação atual da DM baseia-se na sua etiologia e não no tipo de tratamento, portanto, os termos "DM insulino-dependente" e "DM insulino-independente" não devem mais ser utilizados (DSBD, 2015). A classificação proposta pela Organização Mundial da Saúde (WHO) em 2019 inclui as seguintes classes clínicas: DM1, DM2, formas híbridas de diabetes, outros tipos específicos de DM (defeitos genéticos na função das células beta ou na ação da insulina, doenças do pâncreas exócrino, doenças endócrinas, dentre outras condições), diabetes não classificada e hiperglicemia detectada durante a gravidez. Dentre todas, as mais prevalentes e conhecidas são a DM1 e DM2 (WHO, 2019).

A DM1 é caracterizada por ausência total do hormônio insulina devido à destruição das células que a produzem, as células β-pancreáticas e, é mais frequente em crianças e adolescentes, atingindo homens e mulheres de forma equivalente. A presença de DM1 diminui em torno de 13 anos a expectativa de vida (WHO, 2019). A DM2 representa 90 a 95% dos casos de DM e caracteriza-se principalmente pelo comprometimento da ação da insulina, ou seja, RI, resultando em hiperglicemia. Esta é uma doença mais prevalente em adultos e altamente relacionada com estilo de vida não saudável, caracterizado por má alimentação e sedentarismo e consequente excesso de peso (KARALLIEDDE & GNUDI, 2016).

O diagnóstico da DM1 e DM2 considera a presença de um ou mais dos seguintes critérios: glicemia de jejum acima de 125 mg/dL, glicemia de 200 mg/dL duas horas após a ingestão da solução de glicose no teste oral de tolerância à glicose (TOTG), hemoglobina glicada (HbA1c) de 6,5% ou mais e glicemia aleatoria a partir de 200 mg/dL com sintomas de hiperglicemia presentes. Na ausencia dos sintomas, mas com qualquer um desses testes positivos, os mesmos deverão ser repetidos (DSBD, 2019). O diagnóstico da DM1 ocorre geralmente na infância ou adolescência, quando comumente apresenta-se a cetoacidose como primeira manifestação da doença. A ausência ou diminuição severa da secreção de insulina pode ser mensurada pela dosagem do peptídeo C e o processo de auto-destruição pancreática pode ser evidenciado pela presença de autoanticorpos, como os contra a enzima ácido glutâmico descarboxilase (GAD65), antígeno de ilhota-2 (IA-2), transportador de insulina e, ainda, associações com genes envolvidos na resposta

imunológica, como HLA DQ2 e DQ8 (WHO, 2019). É importante mencionar que alguns valores de referencia são considerados para o diagnóstico de uma condição denominada pré-diabetes, que predispõe ao surgimento de DM2, de DCV e complicações crônicas. Estes valores são: glicemia de jejum de 100 a 125mg/dL, glicemia 2 horas após ingestão de glicose no TOTG de 140 a 199mg/dL ou, ainda, HbA1c de 5,7 a 6,4% (SBD, 2019).

O tratamento da DM1 consiste em insulinoterapia, ou seja, reposição de insulina, monitoração constante da glicemia para evitar hipoglicemia, hiperglicemia ou cetoacidose e, cuidado em relação à alimentação e prática de exercício físico (DSBD, 2019). Para a DM2, hábitos alimentares saudáveis e prática de atividade física também são essenciais para manutenção da glicemia, no entanto, neste caso, os pacientes contam com muitos medicamentos hipoglicemiantes orais. Os principais fármacos utilizados são das classes das biguanidas, sulfonilureias, inibidores da dipeptidil peptidase 4 (DPP-4), agonistas do receptor do peptídeo semelhante ao glucagon 1 (GLP-1RA), entre outros (KANECO & NARUKAVA, 2016). A metformina é um dos agentes antidiabéticos mais usados, pertence à classe das biguanidas, que age diminuindo a síntese hepática de glicose e melhorando a sensibilidade dos receptores à insulina, contribuindo então para a diminuição da glicemia e da insulinemia (FONSECA, 2010). No entanto, pacientes em uso desse hipoglicemiante costumam apresentar poliúria, polidipsia, insônia, polifagia, problemas de visão, dor neuropática, dentre outras queixas (CHATURVEDI et al., 2018). As sulfonilureias, como a glibenclamida e a glimepirida, são medicamentos eficazes e de baixo custo, adicionados ao tratamento dos pacientes quando a monoterapia com metformina não é suficiente, mas que também pode ocasionar efeitos adversos como hipoglicemia e ganho de peso (ERIKSSON et al., 2016). Os inibidores da DPP-4 são uma classe de medicamentos que inclui a sitagliptina, vildagliptina, saxagliptina, linagliptina e alogliptina, cujo mecanismo de ação é inibir a enzima DPP-4 e consequentemente aumentar a biodisponibilidade do GLP-1 e do polipeptídeo inibidor gástrico 1 (GIP-1). A enzima DPP-4 é responsável pela degradação de GLP-1 e GIP-1, hormônios da classe das incretinas, secretados pelo intestino em resposta à ingestão de alimentos. Esses hormônios agem aumentando a secreção de insulina, auxiliando a digestão gástrica e aumentando a saciedade e, além disso, GLP-1 inibe a secreção de glucagon (RANKOVIC et al., 2020). Ainda

sobre o GLP-1, outra classe de medicamentos possui seu mecanismo de ação relacionado a esse hormônio, os GLP-1RA. Ao ligar-se especificamente nos receptores, esses medicamentos induzem os efeitos fisiológicos de GLP-1 mencionados anteriormente e ainda resistem à ação das DPP-4. Cabe salientar que apesar dos efeitos benéficos do uso desses medicamentos na manutenção da homeostase glicêmica, alguns efeitos adversos podem surgir, como dor de cabeça, infecções respiratórias, dor e fraqueza muscular com o uso dos inibidores da DPP-4, ou diarreia, náuseas e vômito com o uso dos GLP-1RA (GILBERT & PRATLEY, 2020).

Os inibidores do cotransportador sódio-glicose (SGLT) são considerados medicamentos de segunda ou terceira linha, incluindo a canagliflozina, dapagliflozina, empagliflozina e ertugliflozina, que podem ser utilizados juntamente com qualquer outra classe de medicamentos para tratamento da DM2. Esses medicamentos agem de forma diferente dos demais, sem interferir na secreção e ação da insulina ou outros componentes do metabolismo da glicose, mas sim impedindo a reabsorção de glicose nos túbulos renais. Com isso, diminui-se os níveis de glicose na corrente sanguínea, aumentando a presença de glicose na urina (glicosúria), o que requer atenção devido à tendência a hipoglicemia, infecções no trato urinário e cetoacidose metabólica (TENTOLOURIS et al., 2019).

3.3 Estresse oxidativo

O estresse oxidativo é um quadro caracterizado por uma elevada produção de ERO que excede a capacidade das defesas antioxidantes, ocasionando alterações importantes aos constituintes celulares, como dano ao Ácido desoxidoribonucleico (DNA), alteração na estrutura e função de proteínas, lipídios e carboidratos (CAMPOS & CASADO, 2015). As ERO são produzidas normalmente durante as atividades celulares em diferentes compartimentos, principalmente na mitocôndria, durante a produção de energia. Dentre as ERO mais conhecidas estão as classificadas como radicais livres, como ânion superóxido ($O_2^{-\bullet}$), radical hidroxila ($OH^{\bullet}$) e oxigênio molecular singleto, e a espécie classificada como não radicalar peróxido de hidrogênio (H_2O_2) (ZHANG et al., 2016). Além da produção endógena de ERO, fatores ambientais, como uso de medicamentos (ZHANG et al., 2016), tabagismo, alimentação, poluição do ar, dentre outros, representam fontes exógenas

que contribuem para a instalação ou agravo do estresse oxidativo (CAMPOS & CASADO, 2015).

Para neutralizar os níveis de ERO e proteger as células contra danos, o organismo possui duas formas de defesa antioxidante, que pode ser endógena, a partir da ação de enzimas, como a catalase (CAT), superóxido dismutase (SOD), glutationa peroxidase (GPx) e glutationa S-transferase (GST), ou exógena, feita por compostos como ácido ascórbico, vitamina E, coenzima Q, tocoferol, caroteno, dentre outros (SNEZHKINA et al., 2019). A enzima SOD elimina o $O_2^{-\bullet}$ convertendo-o em H_2O_2 e oxigênio (O_2). A CAT decompõe o H_2O_2 transformando-o em H_2O e O_2. A GPx também atua na decomposição do H_2O_2, utilizando glutationa reduzida (GSH) como cofator, contribuindo ainda, para a menor formação de $OH^{\bullet}$ pela reação de Fenton (WINIARSKA-MIECZAN, 2018). A GST, por sua vez, é considerada uma enzima de segunda linha de defesa antioxidante, capaz de desativar e eliminar radicais livres e os nocivos subprodutos desses radicais, como por exemplo o malondealdeído (MDA), utilizando também GSH como cofator (HAYES & MCLELLAN, 1999).

Uma das consequências do estresse oxidativo é a lipoperoxidação, processo de oxidação de ácidos graxos insaturados das membranas celulares que causa graves alterações na funcionalidade dessas membranas, podendo levar à morte celular. A lipoperoxidação pode ser avaliada a partir dos níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS), onde o MDA, produto formado durante a lipoperoxidação, reage com o ácido tiobarbitúrico, permitindo assim a sua quantificação (BEZERRA et al., 2004). Os nitritos são metabólitos do óxido nítrico (NO) e sua mensuração também pode ser utilizada para avaliar o *status* redox, visto que o excesso de ERO aumenta a degradação de NO (SALAMA et al., 2012). A capacidade de defesa antioxidante pode ser avaliada a partir da atividade das enzimas antioxidantes. Outra forma de avaliar essa capacidade é a quantificação de tiois totais, que permite mensurar as moléculas que possuem o grupo sulfidrila em sua estrutura. Dentre essas moléculas está a GSH, molécula necessária para a desintoxicação exercida pelas enzimas GPx e GST, conforme visto anteriormente (POOLE, 2015).

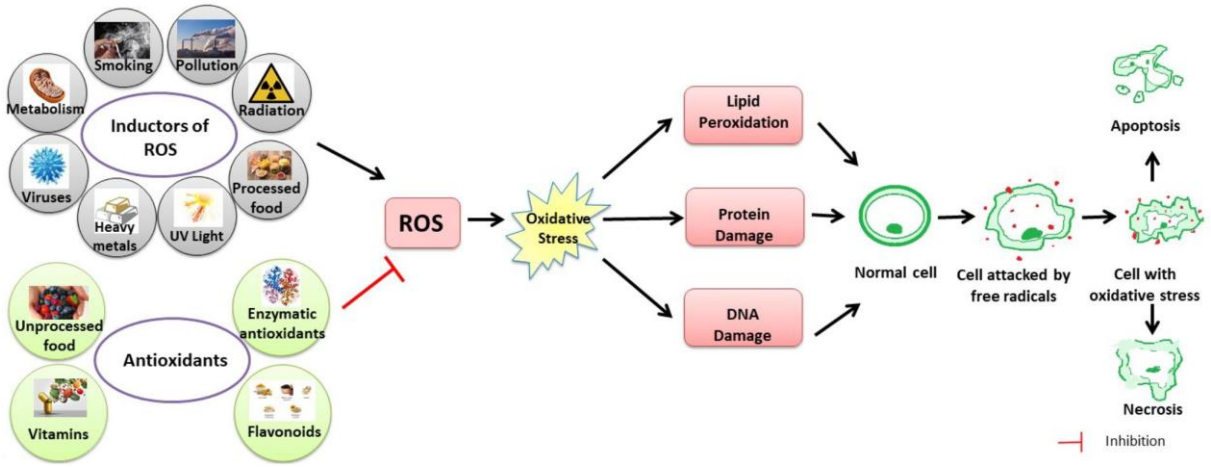

Figura 3: Formação de espécies reativas de oxigênio, defesa antioxidante e consequência do estresse oxidativo (Fonte: Sharifi-Rad et al., 2020).

3.4 Sistema colinérgico

O sistema colinérgico cerebral compreende uma ampla extensão de neurônios responsáveis pela comunicação intercelular e transdução de sinal, envolvendo o neurotransmissor acetilcolina (ACh), seus receptores e enzimas de síntese e degradação (KOUKOULI & CHANGEUX, 2020). A ACh foi um dos primeiros neurotransmissores descritos e é até hoje considerado o principal neurotransmissor do sistema nervoso central (SNC), responsável pelas funções cognitivas, como aprendizado, memória, sono, atenção e emoção, e sistema nervoso periférico (SNP), onde atua como neurotransmissor excitatório, promovendo a contração muscular (FERREIRA-VIEIRA et al., 2016). A síntese de ACh ocorre no citoplasma dos neurônios a partir de colina e acetil-coenzima A (acetil-CoA) pela ação da enzima colina acetiltransferase (ChAT). Logo após a síntese, a ACh produzida é transportada pelo transportador vesicular de ACh (VAChT) para as vesículas sinápticas onde ficam armazenadas até ocorrência de despolarização e sua consequente liberação para a fenda sináptica (ERSKINE et al., 2019).

Na fenda sináptica, para exercer sua função a ACh liga-se a dois tipos de receptores de ACh, classificados em receptores muscarínicos (mAChRs) e receptores nicotínicos (nAChRs). Os mAChRs são receptores do tipo metabotrópicos, associados à proteína G, compreendendo cinco subtipos (M1-M5), sendo que dependendo de qual subtipo se ligar, a ACh terá ação excitatória ou inibitória (SHIN & DIXON, 2015). Os receptores M1, M3 e M5 são do tipo excitatórios, pois possuem a proteína Gq/G11 como ligante para a ACh, promovendo

a partir dessa ligação a ativação da fosfolipase C e formação de diacilglicerol (DAG) e inositol, que contribuem para aumento da concentração de cálcio dentro da célula, e diminui a saída de potássio, ocasionando assim a despolarização e consequente sinapse. Ainda, o receptor M1 é o mais abundante no SNC, sendo distribuído no córtex cerebral, hipocampo e estriado. Já os receptores M2 e M4 são inibitórios, pois ao ligarem-se à proteína Gi/Go inibem a ação da enzima adenilil ciclase e consequentemente de adenosina 3',5'-monofosfato cíclico (cAMP), molécula importante para a sinalização intercelular (JENSEN et al., 2018). Os nAChRs são do tipo ionotrópicos, formados por cinco subunidades, que podem arranjar-se como hetero ou homopentâmeros, e por um canal central permeável à diferentes íons, como sódio, potássio e cálcio. Os nAChRs são categorizados em dois tipos principais, de acordo com seu local inicial onde foram identificados e com sua composição de subunidades. Assim, os nAChRs musculares incluem as subunidades α1, β1, γ, δ e ε, e os nAChRs neuronais incluem nove subunidades α (α2- α10) e três subunidades β (β2- β4) (HAJIASGHARZADEH et al., 2019).

Para finalizar a sinalização colinérgica, duas enzimas são responsáveis por hidrolisar a ACh, a acetilcolinesterase (AChE) e a butirilcolinesterase (BuChE). A primeira hidrolisa exclusivamente a ACh, já a segunda é uma pseudocolinesterase, pois além de ACh hidrolisa colina, butiril, succinilcolina e alguns ésteres alifáticos (FRIEDMAN et al., 2019).

A AChE está distribuída mais abundantemente no SNC, na membrana dos eritrócitos e no músculo esquelético, enquanto a BuChE encontra-se mais predominantemente no plasma sanguíneo (ARAUJO; SANTOS; GONSALVES, 2016). Sabe-se que a BuChE desempenha outras importantes atividades além da hidrólise da ACh, tais como, desintoxicação de pesticidas e outras substâncias químicas tóxicas, inativação de alguns medicamentos e ativação de pró-fármacos. No cérebro, essa enzima hidrolisa de forma compensatória a ACh quando a AChE está esgotada. Além disso, a forma e proporção de hidrólise da ACh ocorre de forma diferente entre essas duas enzimas, onde a etapa limitante da degradação de ACh pela BuChE é a acilação, enquanto pela AChE é a desacilação do substrato. Em relação a taxa de hidrólise, a BuChE é menos eficiente comparada à AChE, no entanto, a BuChE acomoda de forma mais efetiva os inibidores de atividade (ANDRISANO et al., 2018).

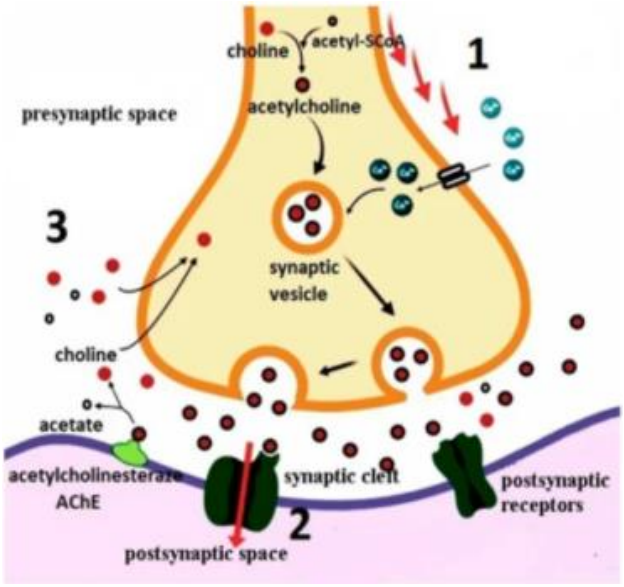

Figura 4: Constituintes do sistema colinérgico (Fonte: Stanciu et al., 2020).

O déficit colinérgico é caracterizado por diminuição da biodisponibilidade de ACh ocasionado por alterações nas atividades ou expressão das enzimas de síntese e degradação, mais especificamente, diminuição da atividade ou expressão da enzima de síntese ChAT e aumento da atividade ou expressão das enzimas de degradação AChE e BuChE (PERRY et al., 1978; BEACH et al., 2000). A disfunção colinérgica e o consequente declínio da função cerebral estão envolvidos na fisiopatologia de diversas patologias como a doença de Alzheimer (DA) (STANCIU et al., 2020), depressão maior e transtorno bipolar (DULAWA & JANOWSKY, 2019). Além disso, a ACh desempenha um importante papel em outros dois processos fisiológicos importantes, a imunidade e a inflamação e, dessa forma, níveis diminuídos de ACh fazem parte da patogenia e agravo de doenças decorrentes de neuroinflamação e/ou alteração no sistema imunológico, como a esclerose múltipla (GATTA et al., 2020). Sendo assim, o uso de inibidores das colinesterases faz parte da terapia de doenças cognitivas, pois aumentam a biodisponibilidade de ACh na fenda sináptica, restaurando as sinapses cerebrais e, consequentemente, melhorando os sintomas cognitivos. No entanto, além dos efeitos adversos, o uso de medicamentos dessa classe não evita a progressão da doença (HAMPEL et al., 2018; GATTA et al., 2020).

Além das alterações enzimáticas, alterações nos receptores M1 e M2, bem como nos receptores nicotínicos α4, α7 e β2, foram encontrados em pacientes com DA (FRANCIS; RAMIREZ; LAI, 2010). Ainda, a diminuição da ligação específica ao

receptor muscarínico M1 foi associada à presença de placas senis em indivíduos sem demência e como esperado, em indivíduos com DA, indicando que a alteração nesse receptor está presente já no estágio inicial de desenvolvimento de demência (POTTER et al., 2011). Assim, a regulação alostérica desses receptores pode vir a ser outra estratégia de tratamento dessas doenças cognitivas (FRANCIS; RAMIREZ; LAI, 2010).

3.5 Alterações cerebrais, oxidativas e colinérgicas relacionadas ao Hipotireoidismo

O hipotireoidismo é uma doença que causa diversas consequências que aumentam o risco de morte e diminuem a qualidade de vida dos indivíduos e, dentre as consequências, comumente pode surgir o comprometimento cognitivo e até mesmo demência grave (RIEBEN et al., 2016). Um estudo de revisão realizado por RITCHIE e YEAP (2015) demonstrou a forte relação do hipotireoidismo com variados transtornos de humor, como depressão, ansiedade e mania, e algumas disfunções cognitivas relacionadas à memória e atenção. E demonstrou ainda, que em muitos casos, a reposição hormonal não foi eficaz em reverter ou melhorar esses problemas (RITCHIE & YEAP, 2015). Cabe salientar que os HT são essenciais para o desenvolvimento neural e permanecem importantes para as funções cerebrais por toda a vida do indivíduo (SALAZAR et al., 2019). Os HT exercem suas funções através da ligação de T3 aos receptores de HT (TR) TRβ1 e TRβ2 expressos no cérebro, que causa uma mudança conformacional no TR e liberação de proteínas ativadoras da transcrição (PESSANHA, 2007). Assim, agem em genes relacionados a diversos processos, como crescimento e diferenciação neural, morfologia dos componentes do SNC, transmissão sináptica, aprendizagem, atenção e memória (SALAZAR et al., 2019).

Dados da literatura apontam principalmente alterações no *status* redox e no sistema colinérgico como principais mecanismos presentes na fisiopatolpogia do hipotireoidismo e na sua relação com as doenças cognitivas (DOMINGUES et al., 2018; NJAN et al., 2020). A relação entre o hipotireoidismo e o estresse oxidativo parece iniciar com o aumento da produção de ERO, facilmente observada em pesquisas utilizando modelo animal de hipotireoidismo. A glândula tireoide possui um sistema gerador de EROs, pois o iodo concentrado da tireoide é oxidado pela

enzima tireoperoxidase utilizando o peróxido de hidrogênio (H_2O_2) como agente oxidante, para então ser incorporado à tireoglobulina (BJORKMAN et al., 1971). Este fato torna a glândula tireoide um dos órgãos mais vulneráveis aos efeitos deletérios do estresse oxidativo.

Em modelo experimental de hipotireoidismo, DOMINGUES et al. (2018) encontraram maior produção de ERO e de TBARS associada à diminuição dos níveis de GSH e da atividade das enzimas antioxidantes GPx e SOD. Além disso, a indução de hipotireoidismo também aumentou a atividade da AChE, enzima que degrada o neurotransmissor ACh, comprometendo assim a transmissão sináptica (DOMINGUES et al., 2018). Em um estudo realizado por TEIXEIRA e colaboradores (2020), um modelo experimental de DA foi caracterizado por aumento da atividade da AChE e alterações na expressão das enzimas de síntese e degradação de ACh, demonstrando assim um déficit colinérgico neste grupo de animais. Associado a isso, os autores observaram também um quadro de estresse oxidativo, observado a partir do aumento dos níveis de ERO e nitritos e diminuição da atividade das enzimas antioxidantes CAT e GPx (TEIXEIRA et al., 2020).

3.6 Alterações bioquímicas, oxidativas e inflamatórias relacionadas à DM2

Pacientes com DM2 possuem predisposição ao surgimento de problemas cognitivos e doenças neurodegenerativas e, dentre os mecanismos envolvidos nessa relação estão principalmente a hiperglicemia, hiperinsulinemia, resistência à insulina, inflamação e estresse oxidativo (MARKOWICZ-PIASECKA et al., 2017; CHENG et al., 2020). É importante destacar que além das ações já conhecidas da insulina relacionada ao metabolismo, ela possui papel fundamental em processos cognitivos, principalmente na região hipocampal do cérebro. Por outro lado, o comprometimento da via de sinalização da insulina, característica principal da DM2, também é observado na região cerebral, e com isso, pode prejudicar a cognição. Além disso, foi demonstrado que esse déficit da sinalização à insulina pode causar atrofia cerebral, diminuição do volume hipocampal e alterações na neurogênese e plasticidade sináptica (SILVA et al., 2019). A RI encontrada em cérebro de animais e indivíduos portadores de DA pode resultar da diminuição da formação, perda e/ou disfuncionalidade dos receptores de insulina. Junto a isso, observa-se uma diminuição da produção de insulina, fazendo com que a DA passasse a ser

chamada de “Diabetes tipo 3” (CHENG et al., 2020). E assim, medicamentos utilizados para o tratamento da DM2 já vêm sendo testados para tratamento e/ou prevenção de doenças neurodegenerativas como a DA e doença de Parkinson (MARKOWICZ-PIASECKA et al., 2017).

Em um estudo realizado por NJAN et al. (2020) utilizando modelo experimental de DM2 induzida por dieta hiperlipídica e dose de estreptozotocina (STZ) (40mg/kg) observou-se alterações em parâmetros de estresse oxidativo em soro, fígado e cérebro, representadas pelos níveis aumentados de nitritos e marcadores de lipoperoxidação e níveis diminuídos de GSH e atividade da enzima CAT. Além disso, a atividade da enzima AChE esteve aumentada nos animais diabéticos. Juntas, essas alterações possivelmente foram responsáveis pelos déficits de memória e aprendizagem demonstrado nos testes comportamentais (NJAN et al., 2020).

A hiperglicemia e a dislipidemia presentes em pacientes com DM2 leva a um quadro de estresse oxidativo a partir de diversos mecanismos, tais como a ativação da via dos poliois, a auto-oxidação da glicose e consequente formação de produtos finais de glicação avançada (AGEs) e o aumento de ácidos graxos livres (AGL). A via dos poliois corresponde à uma via metabólica secundária da glicose em excesso, a qual é convertida em sorbitol, e este último em frutose. Essa conversão ocorre a partir da ação de enzimas específicas que consomem nicotinamida adenina dinucleotideo fosfato (NADPH), molécula necessária para a formação de GSH, essencial para o correto funcionamento de algumas enzimas antioxidantes (PATEL et al., 2016). Os AGEs são formados por uma reação não-enzimática entre glicose, proteínas, lipídeos e ácidos nucleicos, que ao ativarem seus receptores específicos ocasionam inflamação e aumento da produção de ERO, iniciando pela produção do ânion superóxido. Sabe-se ainda, que esses AGEs são encontrados nos emaranhados neurofibrilares e placas de peptídeo β-amiloide, características fisiopatológicas da DA (MARKOWICZ-PIASECKA et al., 2017). Os AGL liberados também aumentam a produção de ERO, que em excesso, levam à oxidação e carbonilação do GLUT4, principal transportador de glicose dos músculos cardíaco e esquelético e do TA, tornando-o disfuncional e impedindo a captação celular de glicose (BODEN et al., 2015).

A DM2 geralmente está associada à obesidade e, o TA de obesos é caracterizado por uma grande liberação e oxidação de AGL, que aumenta os níveis celulares de DAG, acetil-CoA e ceramida. O DAG e a ceramida fosforilam o IRS em serina (Ser), mais especificamente Ser302 e Ser307, locais que afetam negativamente IRS, tornando-o incapaz de ativar a PI3K, prejudicando assim a via de sinalização da insulina (TINKOV et al., 2015). Além disto, há um maior influxo de AGL para o fígado o que estimula a produção de triglicerídeos (TG), lipoproteínas como a lipoproteína de muito baixa densidade (VLDL) e a lipoproteína de baixa densidade (LDL) e diminui os níveis da lipoproteína de alta densidade (HDL), caracterizando uma dislipidemia que poderá levar a aterosclerose e DCV (LAAKSO et al., 2015; PATEL et al., 2016).

Os adipócitos secretam citocinas inflamatórias, como o fator de necrose tumoral α (TNF-α) e interleucina 6 (IL-6), que, por sua vez, provocam a migração de macrófagos para o TA aumentando ainda mais a liberação de citocinas, caracterizando uma inflamação crônica do TA (LAAKSO et al., 2015). Citocinas pró-inflamatórias, como interleucina 1 β (IL-1β), IL-6 e TNF-α comprovadamente induzem inflamação tecidual e/ou sistêmica, comprometendo o funcionamento das células β-pancreáticas, podendo inclusive levar à apoptose celular, comprometendo assim a produção e secreção de insulina, contribuindo para a patogênese da DM2 (REHMAN et al., 2017).

Os adipócitos também secretam as adipocinas, incluindo leptina, adiponectina e resistina para regular o metabolismo sistêmico de lipídios e glicose. A leptina, um hormônio produto do gene obeso (ob) secretado principalmente pelo TA branco, melhora a sensibilidade à insulina e controla o peso corporal, diminuindo a ingestão alimentar e aumentando o gasto energético (CARRASCOSA et al., 2011). No entanto, indivíduos obesos apresentam uma resistência à leptina, devido a alterações no receptor de leptina ou deficiência no transporte deste hormônio pela barreira hemato-encefálica (ROMERO & ZANESCO, 2006). Assim, a alta concentração de leptina, denominada hiperleptinemia, decorrente da sua ineficiente ação, acaba por inibir a captação de glicose estimulada pela insulina (CARRASCOSA et al., 2011). A adiponectina, ao contrário da leptina, tem sua secreção diminuída com o aumento do TA, sendo assim, em situações de menor massa gorda, ela diminui a fosforilação em serina de IRS-1, favorecendo a

fosforilação em tirosina e aumentando a captação de glicose e no hipotálamo estimula o apetite e diminui o gasto energético (CARRASCOSA et al,, 2011). Com a obesidade há uma diminuição nos seus níveis, a hipoadiponectinemia, que está relacionada à disfunção endotelial e RI. ZHAO e colaboradores (2015) mostraram que a administração de adiponectina restaurou a função endotelial e melhorou a sensibilidade à insulina em animais alimentados com alto teor de gordura, mostrando que este hormônio poderá ser um potencial terapêutico para a melhora da RI e prevenção das complicações associadas à DM2

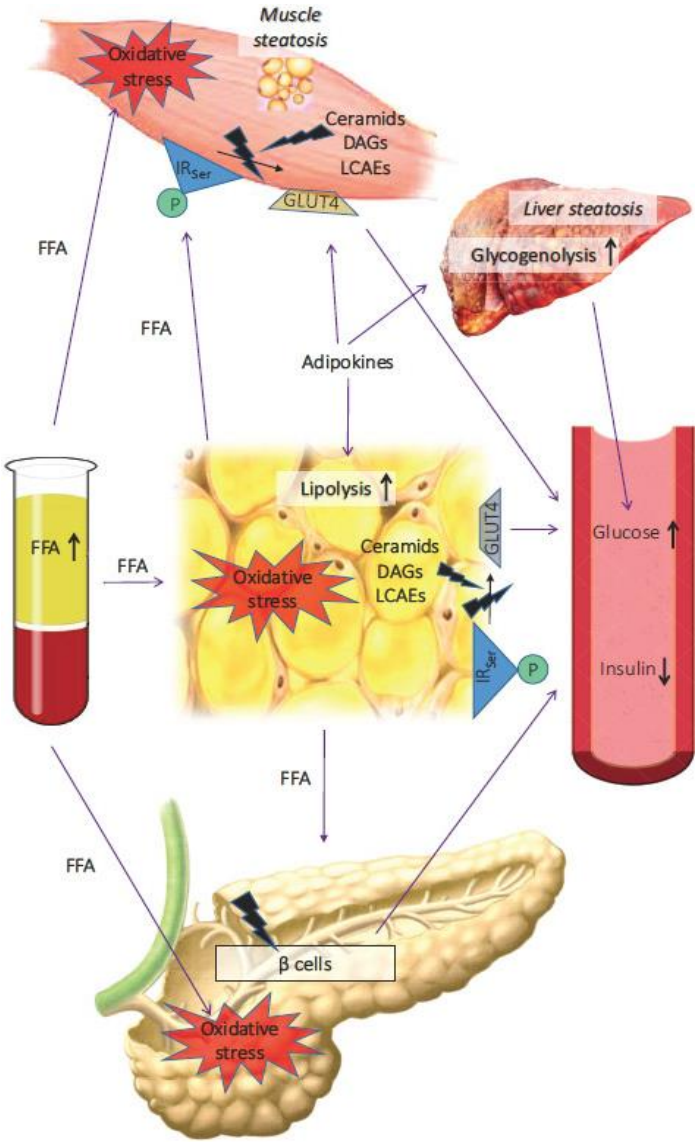

Figura 5: Efeito dos ácidos graxos livres na via de sinalização da insulina e relação com inflamação e estresse oxidativo (Fonte: ZUBRZYCKI et al., 2018)

3.7 Produtos naturais

Considerando a multifatorialidade envolvida no hipotireoidismo e na DM2, a falta de eficácia dos tratamentos disponíveis em reverter os danos já existentes e prevenir a progressão e surgimento das consequências, bem como seus efeitos adversos, torna-se relevante a busca de novas alternativas terapêuticas. Nesse contexto, produtos naturais, como frutos ou compostos isolados, parecem uma alternativa interessante, visto que já demonstraram efeitos benéficos em alterações oxidativas, inflamatórias e colinérgicas, dentre outras (PATEL et al., 2018; MAROYI et al., 2018).

Ao longo da evolução de suas espécies, as plantas e vegetais desenvolveram uma importante capacidade de preservar seu adequado crescimento, desenvolvimento e suas funções fisiológicas frente aos diversos fatores ambientais. Dessa capacidade de defesa resulta a produção de compostos denominados metabólitos secundários (ISAH, 2019). Embora a medicina tradicional seja amplamente utilizada em todo o mundo há milhares de anos, acredita-se que apenas 15% dos constituintes químicos, das 350 mil espécies estimadas de plantas, tenha sido investigado até recentemente (WURTZEL & KUTCHAN, 2016).

Dentre os metabólitos secundários de plantas e vegetais estão os compostos fenólicos, ou polifenois, considerados a classe mais abundante de compostos, que pode ainda ser dividida em três grandes grupos: ácidos fenólicos, flavonoides e não flavonoides. Os ácidos fenólicos são caracterizados por possuir um grupo carboxila ligado ao anel benzeno e derivam de dois compostos fenólicos principais, o ácido benzoico e o ácido cinâmico. Do primeiro, deriva os ácidos hidroxibenzoicos, como o gálico, p-hidroxibenzoico, vanílico e o siríngico. Os derivados do ácido cinâmico são os ácidos hidroxicinâmicos, como o cafeico, ferúlico, sinápico e cumárico (SWALLAH et al., 2020). Os flavonoides compreendem as flavonas, flavanois, flavanonas, isoflavonas e as antocianinas. E por fim, os polifenois não flavonoides abrange as lignanas, os taninos e os estilbenos (DELGADO; ISSAQUI; CHAMMEM, 2019).

Os compostos fenólicos são importantes agentes redutores que transferem elétrons de seus aneis aromáticos para as espécies reativas, estabilizando-as. Além disso, os polifenóis são capazes de inibir as enzimas produtoras de espécies reativas e regular positivamente genes que codificam as enzimas antioxidantes

(DANGLES, 2012). Uma alimentação em longo prazo rica em polifenois tem sido associada à redução de distúrbios neurológicos (DA CUNHA et al., 2016).

O resveratrol pertence ao grupo dos estilbenos, que possui como estrutura básica dois aneis de benzeno ligados por ligação dupla e estão presentes em alimentos como uva, mirtilo, amora, ameixa e vinho, dentre outros (DURAZZO et al., 2019). Os mecanismos de ação envolvidos na proteção antioxidante dos estilbenos parece envolver as vias do fator nuclear derivado do eritroide-2 (Nrf2) e o segundo mensageiro AMPc (REINISALO et al., 2015). Nrf2 é um fator de transcrição que ativa a expressão de genes associados à defesa antioxidante, como gene das enzimas SOD, CAT, GPx e GST, além de modular também a resposta inflamatória e o metabolismo, dentre outras funções (HAHN; DE OLIVEIRA; BOCK, 2017). Com isso, estudos “in vitro”, “in vivo” e clínicos demonstraram o efeito do resveratrol na eliminação de ERO, como o $OH^{\bullet}$, $O_2^{-\bullet}$, H_2O_2 e peroxinitrito ($ONOO^{-}$) (LEONARD et al., 2003; HOLTHOFF et al., 2010; JAVID et al., 2019). Recentemente o extrato de *Paeonia suffruticosa*, contendo dez compostos estilbenos diferentes, dentre eles o resveratrol, demonstrou inibir a atividade das enzimas AChE e BuChE e aumentar os níveis de ACh. Além disso, os compostos preveniram as alterações indicativas de estresse oxidativo, bem como desordem cognitiva indicativa de DA induzida em modelo animal (LIU et al., 2020).

P. cattleianum (Figura 6), conhecida popularmente como “araçá”, “araçá rosa” ou “araçá vermelho” é outra espécie da família Myrtaceae, nativa do Brasil, amplamente cultivada no Sul do país. O araçá possui sabor doce acidulado, forte aroma, grande quantidade de vitamina C e é utilizado na medicina popular para tratamento de distúrbios dolorosos, diarreia, cárie dentária e DM (RIBEIRO et al., 2014). Poucos trabalhos revelam os componentes e as propriedades farmacológicas do araçá, mas já se sabe que seus extratos são ricos em compostos fenólicos, principalmente ácido gálico, e que apresentam atividade antioxidante, antifúngica (CASTRO et al., 2014), antimicrobiana e analgésica (ALVARENGA et al., 2013).

Figura 6: *Psidium cattleianum sabine* (Araçá) (Fonte: Neto et al., 2019).

Recentemente, *P. cattleianum* demonstrou importantes atividades antioxidante, anti-inflamatória, antihiperglicêmica e antilipidêmica em modelo animal de RI (CARDOSO et al., 2018) e síndrome metabólica (SM) (OLIVEIRA et al., 2018; 2020). Além disso, demonstrou prevenir o aumento da atividade das enzimas AChE e BuChE e o comportamento tipo-depressivo em animais com SM (OLIVEIRA et al., 2018, 2020). Um outro fruto rico em polifenois, o *Vaccinium virgatum*, conhecido como blueberry, ou mirtilo no Brasil, também demonstrou proteger contra o estresse oxidativo e déficit colinérgico presentes em animais com episódios de mania, característica do transtorno de humor bipolar (SPOHR et al., 2018). Diversos polifenois demonstram atividade inibitória competitiva e não-competitiva da AChE e BuChE e, possivelmente a ação inibitória decorre da presença de ligações duplas e hidroxilas em suas estruturas, que possibilitam uma forte ligação com aminoácidos do sítio ativo dessas enzimas (KHAN et al., 2018; JABIR; KHAN; TABREZ, 2018).

Os frutos de coloração laranja, vermelha, roxa e azul, possuem em comum a presença de um pigmento pertencente ao grupo dos compostos fenólicos flavonoides, denominadas antocianinas (HE & GIUSTI, 2010). As antocianinas são moléculas derivadas da conjugação de antocianidinas, com açúcar e ácido orgânico, através de reações de hidroxilação, metilação, glicosilação e acilação (ALLAPAT & ALLAPAT, 2020). O consumo de frutas ou suco de frutas ricas em antocianinas, assim como outros polifenois, protege contra o desenvolvimento de doenças crônicas, especialmente doenças associadas ao estresse oxidativo e à inflamação, como doenças cardiovasculares e doenças neurodegenerativas (MATTIOLI et al.,

2020). Ao prevenir o estresse oxidativo, as antocianinas impedem o dano as células β-pancreáticas provocado pelas ERO, prevenindo assim a DM2 (HE & GIUSTI, 2010). Ao administrar extrato de mirtilo (*Vaccinium myrtillus*) em camundongos em apensas 2 semanas, já foi possível detectar antocianinas em fígado, plasma, rins, testículos e pulmões (DEL RIO et al., 2013).

A caracterização fitoquímica do extrato de *P. cattleianum* indicou a presença de 16,72 ± 0,26 mg/g de conteúdo fenólico total, 15,24 ± 2,09 mg/g de flavonoides e 2,48 ± 0,09 mg/g de antocianinas (CARDOSO et al., 2018).

Diante dos dados encontrados na literatura sobre a capacidade antioxidante de compostos fenólicos, bem como de suas ações em parâmetros do sistema colinérgico, tanto o resveratrol, como os extratos de frutos ricos em polifenois, podem ser potenciais alternativas terapêuticas para o hipotireoidismo e DM2, e ainda, para as alterações cerebrais decorrentes dessas doenças.

4 RESULTADOS

Os resultados que fazem parte desta tese estão apresentados sob a forma de um artigo publicado em revista científica e um manuscrito submetido à revista científica. As seções materiais e métodos, resultados, discussão e referências encontram-se no próprio artigo e manuscrito e representam a íntegra deste estudo. Os ítens discussão e conclusões apresentam uma interpretação final sobre o trabalho, artigo e manuscrito em geral. As referências são apenas de citações que aparecem nos itens introdução e referencial teórico da tese. O artigo e o manuscrito estão estruturados de acordo com as revistas as quais foram publicados ou submetidos.

4.1 Artigo 1

Neuroprotection elicited by resveratrol in a rat model of hypothyroidism: Possible involvement of cholinergic signaling and redox status

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Neuroprotection elicited by resveratrol in a rat model of hypothyroidism: Possible involvement of cholinergic signaling and redox status

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ABSTRACT

Both the cholinergic pathway and oxidative stress are important mechanisms involved in the pathogenesis of hypothyroidism, a condition characterized by low levels of thyroid hormone that predispose the patient to brain dysfunction. Phenolic compounds have numerous health benefits, including antioxidant activity. This study evaluates the preventive effects of resveratrol in the cholinergic system and redox status in rats with methimazole-induced hypothyroidism. Hypothyroidism increases acetylcholinesterase (AChE) activity and density in the cerebral cortex and hippocampus and decreases the α7 and M1 receptor densities in the hippocampus. Hypothyroidism also increases cellular levels of reactive oxygen species (ROS) and thiobarbituric acid reactive substances (TBARS), but reduces total thiol content, and catalase and superoxide dismutase activities in the serum. In the cerebral cortex and hippocampus, hypothyroidism increases the levels of ROS and nitrites. In this study, resveratrol (50 mg/kg) treatment prevents the observed increase in AChE in the cerebral cortex, and increases the protein levels of NeuN, a marker of mature neurons. Resveratrol also prevents changes in serum ROS levels and brain structure, as well as the levels of TBARS, total thiol content, and serum catalase enzyme activity. These collective findings suggest that resveratrol has a high antioxidant capacity and can restore hypothyroidism-triggered alterations related to neurotransmission. Thus, it is a promising agent for the prevention of brain damage resulting from hypothyroidism.

1. Introduction

Hypothyroidism is a disorder of the thyroid gland, characterized by low levels of thyroid hormone (TH), and affects approximately 10% of the population (Biondi and Cooper, 2019). Current treatment methods aim to maintain adequate levels of TH through oral replacement of thyroxine (T4), which is metabolically transformed to its active state, triiodothyronine (T3). However, when to begin treatment and the treatment dose remain contentious, given the complexity of this disorder and the different types of hypothyroidism (Mateo and Hennessey, 2019).

TH is important in regulating neurological functions at all stages of life; it modulates critical roles, including neurogenesis, glial evolution, proliferation of dendritic outspread, cognitive performance, and brain metabolism energy (Salazar et al., 2019; Salami et al., 2019) through the

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regulation of the expression of genes in brain tissue. Several fundamental genes for brain formation and function are dependent on the TH. These include genes related to myelination, structure, and the biochemistry of brain mitochondria and transcription factors (Bernal, 2002). Therefore, TH affects cell maturation in areas with considerable postnatal neurogenesis, including the hippocampus (Salami et al., 2019), and TH deficiencies can thus lead to brain dysfunction that causes behavioral and cognitive disorders. In the early stages of life, this impairment can be irreversible (Poterfield, 1994). The relationship between maternal TH insufficiency and neurodevelopment in children has been studied. These findings indicate an association of the insufficiency with cognitive deficits, attention deficit hyperactivity disorder, and increased susceptibility to seizures (O'Shaughnessy et al., 2019).

Several brain functions depend on the correct functioning of the cholinergic system, which comprises the action and bioavailability of the neurotransmitter and neuromodulator acetylcholine (ACh), as well as its receptors and their synthesis and degradation enzymes (Piccioto et al., 2012). ACh is one of the primary neurotransmitters that regulate states of sleep and wakefulness, locomotor activity, and cognitive processes such as memory and learning (Colangelo et al., 2019). It acts by binding two classes of receptors: nicotinic receptors (nAChRs) and muscarinic receptors (mAChRs). The α7 nAChRs are the main receptors found in the brain of rodents and are responsible for the cognitive functions of central nervous system (CNS) (Fukunaga and Yabuki, 2018). mAChRs are involved in numerous important functions, including memory, learning, and emotions, The M1 subtype represents approximately 50% of all mAChRs in the CNS (Ventura et al., 2010). Therefore, cholinergic dysfunction is present in the pathogenesis of several diseases, including Alzheimer's disease (Pákáski and Kalmán, 2008), Parkinson's disease (Nardone et al., 2017), mood disorders (Dagytė et al., 2011; Yong and Dulcis, 2015), and even hypothyroidism, where the presence of cognitive dysfunction has also been demonstrated (Xu et al., 2019).

In addition to cholinergic dysfunction, oxidative stress, which is characterized by a high production of reactive species sufficient to exceed the detoxification capacity of antioxidant defenses (Campos and Casado, 2015), is an important mechanism in the development of several diseases, including several types of cancer (Nowsheen et al., 2009), metabolic disorders (Cardoso et al., 2018), various neurodegenerative diseases (Radi et al., 2014), and hypothyroidism (Jena, 2015). Therefore, compounds that restore cholinergic dysfunction and oxidative stress are important agents for the prevention or treatment of these diseases.

Phenolic compounds are plant secondary metabolites characterized by an aromatic ring with at least one hydroxyl group. They are classified primarily as flavonoids or non-flavonoids, and have been linked to improved health, disease prevention and treatment (Durazzo et al., 2019). Among the class of non-flavonoid compounds, resveratrol (3,4', 5-trihydroxystilbene) is synthesized by various plants, including fruits, peanuts, cocoa, and grape skins, in response to bacterial and fungal attack (Castaldo et al., 2019). Resveratrol can inhibit AChE and displays pronounced antioxidant capacity in an animal model of neurodegenerative disorders (Lu et al., 2013). Preclinical studies have demonstrated that this polyphenol exerts beneficial effects on glucose and lipid homeostasis, thereby preventing insulin resistance, diabetes, and dyslipidemia (Chaplin et al., 2018). Additionally, resveratrol have been shown to modulate the expression of several genes related to oxidative stress and inflammation (Truong et al., 2018). Furthermore, this phenolic compound acts in pathways related to numerous signaling molecules, such as sirtuin type 1 (SIRT1), caspases, nuclear factor kappa-B (NF-kB), nuclear factor-erythroid 2-related factor 2 (Nrf2), intercellular adhesion molecule (ICAM), vascular cell adhesion molecule (VCAM), insulin-like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), cycloxygenase 2 (COX-2), and Kelch-like ECH–associated protein 1 (Truong et al., 2018, Singh et al., 2017). The ability of resveratrol to interact with multiple targets, such as kinases, receptors, and signaling molecules, allows it to exert the pleiotropic behavior (Singh et al., 2017). In this context, this work aims to evaluate the preventive effects of resveratrol on changes in the cholinergic pathway and oxidative stress parameters in rats with methimazole-induced hypothyroidism.

2. Materials and methods

2.1. Chemicals

Methimazole (MMI) (approximately 99% pure), 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA), trichloroacetic acid (TCA), malondialdehyde (MDA), and Coomassie brilliant blue G were obtained from Sigma-Aldrich (USA). Resveratrol (approximately 99% pure) was obtained from Fagron (Belgium).

2.2. Animals

Thirty adult male Wistar rats (50–60 days of age; 250–300 g) were obtained from the Central Animal House of the Federal University of Pelotas. The animals were maintained under appropriate conditions (controlled temperature of 22 ± 1 °C and a 12 h light/dark cycle) and received commercial standard chow and water or MMI solution *ad libitum*. All procedures were carried out according the "Guide for the Care and Use of Laboratory Animals" (US National Institutes of Health publication no. 85-23, revised 1996). The current protocol has been approved by the Animal Ethics Committee of the Institution (CEEA 12594-2020).

2.3. Experimental hypothyroidism model and drug treatment

All animals were arbitrarily assigned before starting treatment and the experimenters were blinded to the group allocation. The animals were randomly divided into three groups (n = 10 per group): Control/Vehicle (CT/V), Hypothyroidism/Vehicle (Hypo/V), and Hypothyroidism/Resveratrol (50 mg/kg; Hypo/Resv). Hypothyroidism was induced by the MMI antithyroid drug, which was administered *ad libitum* in the drinking water at a concentration of 20 mg/100 mL over 30 days, as previously described (Baldissarelli et al., 2016, 2017).

Resveratrol was freshly prepared in saline and administered orally by gavage once daily between 10 a.m. and 11 a.m. until the end of the experiment. The administered volume did not exceed 0.1 mL/100 g body weight. The dose of resveratrol administered was determined as previously described by Gocmez et al. (2016). The animals of the CT/V and Hypo/V groups received saline by gavage instead.

2.4. Sample collection

After 30 days, the animals were sacrificed and total blood was collected to obtain serum and plasma. The cerebral cortex and hippocampus were collected for neurochemical and molecular analyses.

2.5. Measurement of T3 and T4 levels

The induction of hypothyroidism by MMI was confirmed using the AxSYM® (Abbott Laboratories, USA) enzyme immunoassay, according to suppliers' instructions. The total levels of T3 and T4 were measured and expressed as ng/mL and μg/mL plasma, respectively.

2.6. Acetylcholinesterase (AChE) activity

AChE activity was assessed in the cerebral cortex and hippocampus as previously described by Ellman et al. (1961), and expressed as μmol AcSCh/h/mg protein. Initially, the homogenate was incubated with DTNB (10 mM) and phosphate buffer (100 mM, pH 7.5) for 2 min at 27 °C. Thereafter, acetylthiocholine (AcSCh) (0.8 mM) was added to initiate the enzymatic reaction. The hydrolysis of AcSCh generates

thiocholine that reacts with 5,5′-dithio-bis-2-nitrobenzoic acid reagent (DTNB) resulting in the generation of 5,5′-dithiobis-2-nitrobenzoate (TNB). TNB quantification was performed using a microplate reader (SpectraMax 190, Molecular Devices, USA) at 412 nm at 30 s intervals for 2 min at 27 °C. The protein content was assessed using the standard *Coomassie* blue method developed by Bradford (1976).

2.7. AChE, cholinergic receptors, neuronal nuclei (NeuN), and glial fibrillary acidic protein (GFAP) density

The density of AChE, α7-nAChR, M1-mAChR, NeuN, and GFAP was evaluated in the cerebral cortex and hippocampus, as previously described by Reichert et al. (2018). The brain samples were homogenized in cold radioimmunoprecipitation assay buffer (RIPA buffer) with 220 mM phenylmethanesulfonyl fluoride (PMSF, 1:1000), a protease inhibitor, and transferred to the nitrocellulose membranes (Amersham Biosciences, UK). After blocking, the membranes were incubated at 4 °C overnight with the primary antibodies against AChE, α7-nAChR, and M1-mAChR (1:800; Santa Cruz Biotechnology, USA), NeuN (1:1000; Merck Millipore, USA), and GFAP (1:1000; Cell Signaling Technology, USA), followed by incubation with the secondary antibodies (1:10000; Thermo Scientific, USA) at room temperature for 90 min. The membranes were then incubated with enhanced chemifluorescence substrate (Amersham Biosciences, UK) and analyzed using the Amersham Imager 600 (GE Healthcare Life Sciences, USA). Additionally, the membranes were re-probed and tested for β-actin immunoreactivity as a control to assess the protein concentration as previously described (Rebola et al., 2003). The protein concentration was determined using the BCA assay kit (Sigma Chemical Corporation, USA).

2.8. Oxidative stress parameters

The serum parameters of oxidative stress that were evaluated included levels of reactive oxygen species (ROS), thiobarbituric acid reactive substances (TBARS), total thiol content, and the activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT). ROS and nitrite levels were also measured in the cerebral cortex and hippocampus. For this, the brain structures were homogenized using the sodium phosphate buffer (pH 7.4) containing KCl. Subsequently, the homogenates were centrifuged at 800 x *g* for 10 min at 4 °C, and the supernatant was used for the downstream analyses. ROS levels were assessed on the basis of the oxidation of dichlorofluorescein diacetate (DCFH-DA) to fluorescent 2′, 7′-dichlorofluorescein (DCF), and the intensity was measured at 488–525 nm 30 min after adding DCF in the medium. Results were expressed as μmol DCF/mg protein (Ali et al., 1992). TBARS levels were assessed as described previously (Esterbauer and Cheeseman, 1990), where the samples were first mixed with 15% trichloroacetic acid (TCA) and centrifuged. Then, the supernatant was mixed with 0.67% thiobarbituric acid (TBA) in water and incubated at 100 °C for 30 min. TBARS levels were determined through measuring the absorbance at 535 nm and expressed as nmol TBARS/mg protein. Total thiol content was measured according to the methodology described by Aksenov and Markesbery (2001), which is based on the reduction of DTNB to a yellow placement derivative (TNB) in the presence of thiols, and the absorbance was measured at 412 nm. For this, the homogenates were mixed with PBS buffer, pH 7.0, containing EDTA. The reaction was initiated through the addition of DTNB, and the results were expressed as nmol TNB/mg protein. SOD activity was measured as previously described by Misra and Fridovich (1972). This assay is based on the inhibition of adrenaline autoxidation, which is dependent on superoxide in adrenochrome. The intermediate in this reaction is superoxide, which is eliminated by SOD and is assessed by measuring the absorbance using a microplate reader (SpectraMax 190, Molecular Devices, USA) at 480 nm and expressed as units/mg protein. CAT enzyme activity was measured by assessing the decomposition of H_2O_2 monitored for 180 s at 240 nm and expressed as units/mg protein (Nelson and Kiesow, 1972). Nitrite concentration was determined as previously described by Stuehr and Nathan (1989). For this, the supernatants were subjected a colorimetric reaction with Griess reagent upon addition of sulphanilamide prepared in 5% phosphoric acid. Thereafter, the samples were mixed with N-(1-naphthyl) ethylenediamine dihydrochloride and incubated for 10 min in the dark. The absorbance was measured at 540 nm and the amount of nitrite was expressed as μmol nitrite/mg protein."

2.9. Statistical analysis

Data were analyzed using the software GraphPad Prism 5.0 (GraphPad Software, USA) using one-way ANOVA and the Tukey *post hoc* test, with $P < 0.05$ representing a significant difference.

3. Results

3.1. Plasma T3 and T4 levels

Administration of MMI for 30 days significantly reduced the levels of T3 ($F_{(2,26)} = 369.1$; $P < 0.001$) and T4 ($F_{(2,22)} = 792.8$; $P < 0.001$), indicating the establishment of hypothyroidism in the rats. The use of resveratrol during this period was not sufficient to prevent this decrease (Table 1).

3.2. AChE activity and density

Fig. 1 depicts AChE activity (A) and density (B). Increased AChE activity was observed in the cerebral cortex ($F_{(2,12)} = 9.02$; $P < 0.01$) and hippocampus ($F_{(2,12)} = 11.54$; $P = 0.001$) provided by rats with hypothyroidism. On the other hand, resveratrol was able to prevent this increase. Similarly, AChE density was increased in both the cerebral cortex ($F_{(2,6)} = 16.56$; $P < 0.01$) and hippocampus ($F_{(2,6)} = 39.17$; $P < 0.001$) of the two groups of animals that received MMI to induce hypothyroidism.

3.3. Cholinergic receptors, NeuN and GFAP densities

Fig. 2 shows the α7-nAChR (A) and M1-mAChR (B) densities in the cerebral cortex and hippocampus. There was no significant change in α7-nAChR density in the cerebral cortex between the groups ($F_{(2,6)} = 0.61$; $P > 0.05$). However, in the hippocampus of the Hypo/V and Hypo/Resv groups ($F_{(2,6)} = 42.17$; $P < 0.001$) is evident a decreasing values of α7-nAChR density. Decreased M1-mAChR density was evident in the hippocampus in the Hypo/V and Hypo/Resv groups ($F_{(2,6)} = 16.60$; $P < 0.01$), but the density in the cerebral cortex was not significantly different ($F_{(2,6)} = 0.28$; $P > 0.05$). Fig. 3 depicts the NeuN (A) and GFAP (B) densities in the hippocampus. An increase in the NeuN density was evident in the group that received resveratrol (Hypo/Resv) compared with the CT/V and Hypo/V groups ($F_{(2,6)} = 87.58$; $P < 0.001$). GFAP density was not significantly different between the groups ($F_{(2,6)} = 1.09$; $P > 0.05$).

3.4. Oxidative stress parameters

Table 2 summarizes the results for the oxidative stress parameters in

Table 1
Effects of resveratrol treatment on plasma thyroid hormones levels of rats with MMI-induced hypothyroidism.

	CT/V	Hypo/V	Hypo/Resv
T3 (ng/dL)	1.19 ± 0.03	0.49 ± 0.01***	0.48 ± 0.01***
T4 (μg/dL)	6.53 ± 0.18	0.42 ± 0.01***	0.41 ± 0.003***

Data are expressed as mean ± S.E.M. (n = 7–10). *** denotes $P < 0.001$ as compared with the CT/V group. One-way ANOVA followed by Tukey post hoc test was used. CT, control; V, vehicle; Hypo, hypothyroidism; Resv, resveratrol.

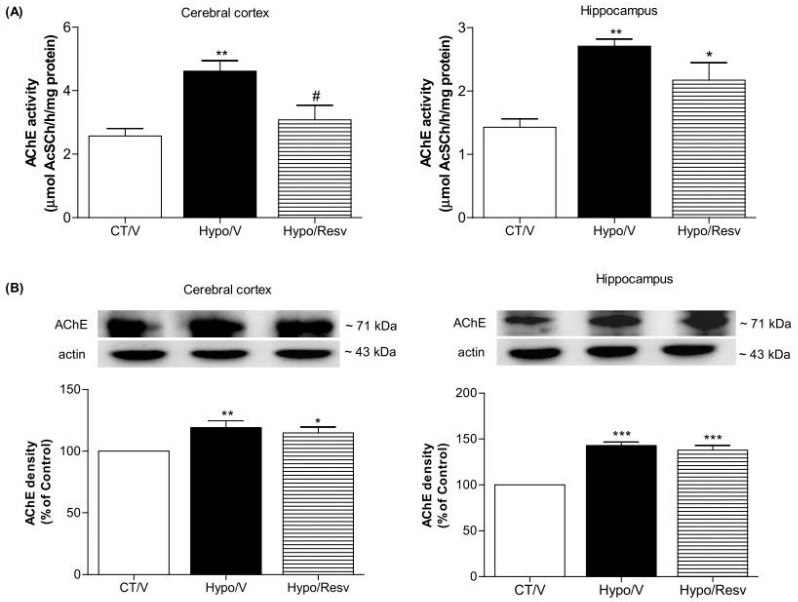

Fig. 1. Effect of resveratrol (50 mg/kg) on AChE activity (A) and AChE density (B) in the cerebral cortex and hippocampus of rats with MMI-induced hypothyroidism. Data are expressed as mean ± S.E.M. (n = 3–6). ***denotes $P < 0.001$, ** denotes $P < 0.01$, and * denotes $P < 0.05$ as compared with the CT/V group. # denotes $P < 0.05$ as compared with the Hypo/V group. Data were analyzed using One-way ANOVA followed by Tukey post hoc test. Abbreviations: CT, control; V, vehicle; Hypo, hypothyroidism; and Resv, resveratrol.

serum. Resveratrol prevented modifications caused by hypothyroidism, with decreased levels of ROS ($F_{(2,21)} = 9.01$; $P = 0.001$) and TBARS ($F_{(2,23)} = 8.94$; $P = 0.001$), and increased total thiol content ($F_{(2,22)} = 6.22$; $P < 0.05$) and CAT activity ($F_{(2,21)} = 5.70$; $P = 0.01$). SOD activity was decreased in both the Hypo/V and Hypo/Resv groups ($F_{(2,19)} = 13.96$; $P < 0.001$).

Table 3 presents the findings of the redox status in the cerebral cortex and hippocampus. We observed an increase of ROS levels, both in cerebral cortex ($F_{(2,12)} = 11.92$; $P < 0.01$) and hippocampus ($F_{(2,10)} = 13.86$; $P = 0.001$). Resveratrol was effective at preventing this increase. Similarly, nitrite levels were increased in the cerebral cortex of rats with hypothyroidism ($F_{(2,12)} = 5.64$; $P = 0.01$) and hippocampus ($F_{(2,12)} = 5.89$; $P = 0.01$). Opposite, treatment with resveratrol was not sufficient to prevent this increase.

4. Discussion

The TH is involved in many vital processes in brain development and function. Several studies have demonstrated the deleterious effects of hypothyroidism on the physiology (Nam et al., 2018; Torres-Manzo et al., 2018; Seymen et al., 2020) and function (Gilbert and Paczkowski, 2003 Gilbert, 2004) of the hippocampus, as well as in cognitive function and mood disorders in animal models (Hasegawa and Wada, 2013; Umezu et al., 2019) and clinical studies (Wekking et al., 2005; Mowla et al., 2011; Wu et al., 2013) with this disease. Moreover, hypothyroidism is related to changes in the development and activity of structural components of the cerebral cortex (Martinez et al., 2009; Santalucía et al., 2006). These alterations in hippocampus and cerebral cortex can impair spatial learning and memory (Zhang et al., 2018).

Since ACh is one of the most important neurotransmitters, knowledge of the activity and expression of the enzymes involved in its degradation is relevant to understanding the mechanisms involved in brain changes. Presently, we evaluated AChE activity and demonstrated increases in the cerebral cortex and hippocampus of rats with hypothyroidism. This increased activity negatively affects the availability of the neurotransmitter in the synaptic cleft, which causes cholinergic dysfunction (Muramatsu et al., 2018). In this study, we observed that resveratrol was effective at preventing the increased activity in the cerebral cortex of animals. We thus suggest that resveratrol could be an important neuromodulator.

Beyond enzyme activity, the expression of AChE could be a method to assess cholinergic function. We observed that the induction of hypothyroidism increased AChE expression in both the brain structures we evaluated. A prior study (Cuadrado et al., 2003) demonstrated that T3 acts directly by reducing the transcription rate of the *HuD* gene and that, conversely, hypothyroidism produces an overexpression of *HuD* in the brains of rats. *HuD* is one of four different genes in the embryonic lethal/abnormal visual system (ELAV)/Hu RNA-binding protein family. *HuD* is essential for the development and maintenance of the nervous system. The gene encoding *HuD* is specifically expressed in neuronal tissues and neuroendocrine tumors and has a high affinity for AChE mRNA, the binding of which stimulates the expression of AChE (Deschênes-Furry et al., 2007). Considering this finding, it is possible that T3 deficiency increases the expression of AChE through *HuD*-mediated gene regulation, resulting in a cholinergic deficit. Furthermore, we could speculate that the use of resveratrol did not prevent changes in AChE expression, since this compound did not reverse the serum levels of T3 and T4. Additionally, resveratrol seems to modulate only the AChE enzyme activity, since the use of this phenolic compound led to a decrease in its enzyme activity, even without affecting AChE protein expression level.

MMI-induced hypothyroidism is caused by oxidative stress, involving increased levels of ROS and a corresponding increase in lipid peroxidation, alterations in the ratio of the reduced and oxidized

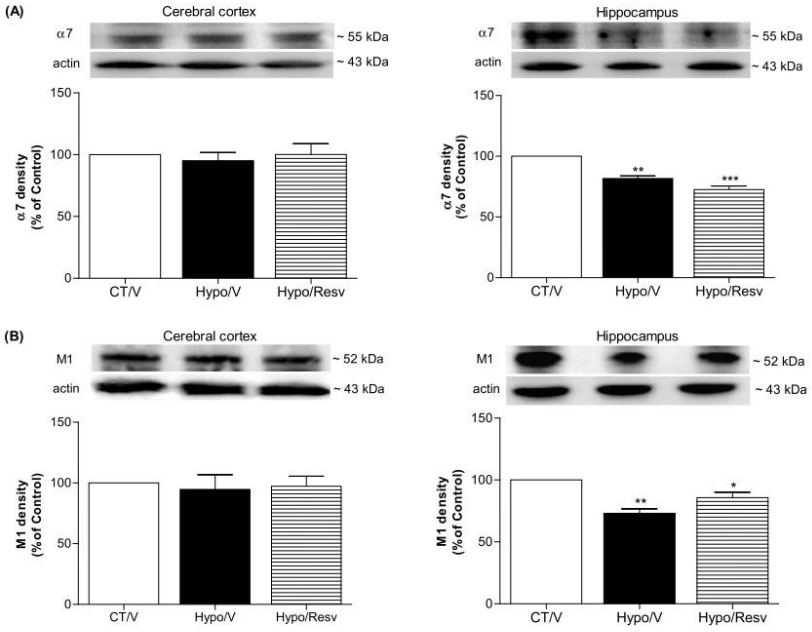

Fig. 2. Effect of resveratrol (50 mg/kg) on α7 (A) and M1 (B) receptor densities in the cerebral cortex and hippocampus of rats with MMI-induced hypothyroidism. Data are expressed as mean ± S.E.M. (n = 3). *** denotes $P < 0.001$, ** denotes $P < 0.01$, and * denotes $P < 0.05$ as compared with the CT/V group. Data were analyzed using one-way ANOVA followed by Tukey post hoc test. Abbreviations: CT, control; V, vehicle; Hypo, hypothyroidism; and Resv, resveratrol.

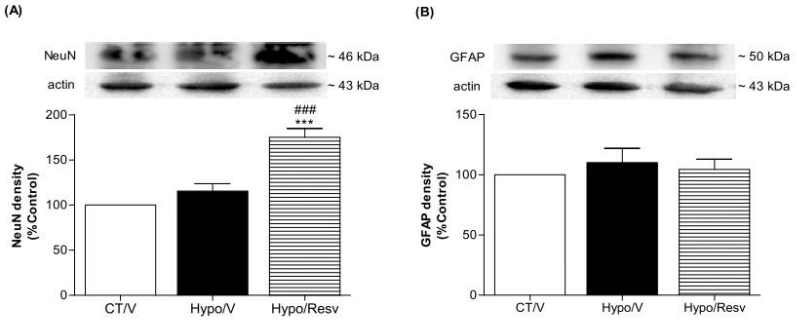

Fig. 3. Effect of resveratrol (50 mg/kg) on NeuN (A) and GFAP (B) densities in hippocampus of rats with MMI-induced hypothyroidism. Data are expressed as mean ± S.E.M. (n = 3). *** denotes $P < 0.001$ as compared with CT/V group. ### denotes $P < 0.001$ as compared with the Hypo/V group. Data were analyzed using One-way ANOVA followed by Tukey post hoc test. Abbreviations: CT, control; V, vehicle; Hypo, hypothyroidism; and Resv, resveratrol.

glutathione couple, and a reduction of CAT activity (Cano-Europa et al., 2010). In this study, we demonstrated an increase in ROS levels in the cerebral cortex, hippocampus and serum, as well as in TBARS levels and a reduction of serum CAT activity. Resveratrol, an important antioxidant compound, was able to prevent these alterations in oxidative stress parameters. It is worth-mentioning that resveratrol is able to cross the blood-brain barrier and upregulate the isoforms of antioxidant enzymes. Also, it increases the enzymatic activity, especially of CAT, which contributes to a reduction in oxidative damage and neurodegeneration (Mokni et al., 2007). Other authors used quercetin, an important flavonoid with antioxidant properties, to demonstrate its effectiveness in reverting the increase in TBARS and the reductions of SOD and CAT activities in animals with MMI-induced hypothyroidism (Baldissarelli et al., 2016).

It is well known that gene expression process and signal transduction pathways are affected by many parameters, such ROS. Particularly, it was demonstrated that oxidative stress increases the activity and expression of the AChE enzyme. The ROS accumulation causes damage to the cell membrane (lipid peroxidation), exposing the AChE active site and, consequently, increasing its activity (Schmatz et al., 2009). In addition, the presence of hydrogen peroxide (H_2O_2) increases the expression of AChE through mitogen-activated protein kinases (AMPK)

Table 2
Effects of resveratrol treatment on oxidative stress parameters in serum of rats with MMI-induced hypothyroidism.

	CT/V	Hypo/V	Hypo/Resv
ROS levels (μmol DCF/mg protein)	0.382 ± 0.037	0.506 ± 0.026 *	0.344 ± 0.029$^{\#\#\#}$
TBARS levels (nmol/mg protein)	0.118 ± 0.011	0.216 ± 0.021 **	0.139 ± 0.019$^{\#}$
SH levels (nmol TNB/mg protein)	3.480 ± 0.283	2.160 ± 0.162 **	3.161 ± 0.379$^{\#}$
SOD activity (U/mg protein)	1.757 ± 0.103	1.050 ± 0.101 ***	1.223 ± 0.076 **
CAT activity (U/mg protein)	0.238 ± 0.031	0.133 ± 0.012 *	0.2346 ± 0.026$^{\#}$

Data are expressed as mean ± S.E.M. (n = 10). *** denotes $P < 0.001$, ** denotes $P < 0.01$, and * denotes $P < 0.05$ as compared with the CT/V group. $^{\#\#\#}$ denotes $P < 0.001$ and $^{\#\#}$ denotes $P < 0.01$ as compared with the Hypo/V group. One-way ANOVA followed by Tukey post hoc test was used. CT, control; V, vehicle; Hypo, hypothyroidism; Resv, resveratrol.

Table 3
Effects of resveratrol treatment on oxidative stress parameters in the cerebral cortex and hippocampus of rats with MMI-induced hypothyroidism.

	CT/V	Hypo/V	Hypo/Resv
Cerebral cortex			
ROS levels (μmol DCF/mg protein)	450.1 ± 31.01	758.4 ± 68.41 **	449.9 ± 31.92$^{\#\#}$
Nitrite levels (μmol nitrite/mg protein)	3.860 ± 0.103	4.880 ± 0.183 *	4.680 ± 0.334 *
Hippocampus			
ROS levels (μmol DCF/mg protein)	297.6 ± 12.60	521.2 ± 34.36 **	328.0 ± 36.99$^{\#\#}$
Nitrite levels (μmol nitrite/mg protein)	3.20 ± 0.130	4.50 ± 0.217 *	4.00 ± 0.394 *

Data are expressed as mean ± S.E.M. (n = 5–6). ** denotes $P < 0.01$ and * denotes $P < 0.05$ as compared with the CT/V group. $^{\#\#}$ denotes $P < 0.01$ as compared with the Hypo/V group. One-way ANOVA followed by Tukey post hoc test was used. CT, control; V, vehicle; Hypo, hypothyroidism; Resv, resveratrol.

(Zhang et al., 2008). Studies using resveratrol analogues demonstrated that these compounds inhibited AChE activity and had high antioxidant capacity, two important effects for the treatment of cognitive impairment (Lu et al., 2013; Pan et al., 2014; De Assis et al., 2020). In addition, resveratrol reportedly improves cognitive deficits, possibly by regulating AChE activity and restoring redox status and inflammatory parameters (Tiwari and Chopra, 2013; Ma et al., 2020). Furthermore, Tian et al. (2016) demonstrated that resveratrol increased the levels of synaptic plasticity markers, such as the presynaptic vesicle protein synaptophysin (SYN) and the growth-associated axonal protein-43 (GAP-43) in hippocampus of diabetic rats. Additional parameters involved in the differentiation, survival, and other aspects of hippocampal neural plasticity, such as brain-derived neurotrophic factor (BDNF), phosphorylated extracellular regulated kinase (pERK) and phosphorylated cyclic adenosine monophosphate response element-binding protein (pCREB), are also found to be restored upon resveratrol treatment (Dias et al., 2016). Moreover, Serra et al. (2019) demonstrated that resveratrol is effective in preserving and/or enhancing nervous tissue neuroplastic potential in brain cortex of rats submitted to the hypoperfusion/reperfusion challenge, modulating BDNF-trkB system, polysialylated-neural cell adhesion molecule (PSA-NCAM) and activity-regulated cytoskeleton-associated (Arc) protein levels. Other polyphenols and their naturally-derived derivatives have shown promise in the treatment of different pathologies that require high levels of ACh (Baldissarelli et al., 2017; Guo et al., 2019).

The increase in activity and density of AChE in the groups that received MMI to induce hypothyroidism might be related to the oxidative damage, especially peroxidation of membrane lipids. Therefore, although resveratrol has protective effect against oxidative stress, its use was not effective in preventing the increase in the expression of AChE. Therefore, we propose that AChE activity and expression are independent parameters, and the effect of resveratrol treatment could be explained as a consequence of the interaction of this compound with the active site of AChE enzyme, as also demonstrated upon using the analogs of resveratrol (De Assis et al., 2020). Similarly, other researchers also demonstrated that there is no correlation between AChE activity and expression in both clinical and preclinical studies (Zhang et al., 2011; Işık et al., 2017; Leal et al., 2017).

Drug-induced hypothyroidism has been associated with selective oxidative stress in the hippocampus and amygdala of hypothyroid rats, with the involvement of endothelial nitric oxide synthase (Mancini et al., 2016). Herein, we observed increases in ROS and nitrite levels in the cortex and hippocampus. The excess ROS could increase the degradation of nitric oxide (NO), in addition to impairing the enzyme activity and bioavailability of cofactors essential to produce NO. Thus, high levels of NO metabolites such as nitrites can indicated reduced of the bioavailability of NO, which can cause endothelial dysfunction, among other consequences (Parodi et al., 2007).

Given the importance of receptors for the proper functioning of the cholinergic system, knowledge about its availability and functionality is relevant. The decreased M1 density observed in animal models of neurodegenerative disease and in elderly rats has been associated with memory and learning disorders. These changes were restored after the use of a natural compound from the saponin family (Hu et al., 2005). A positive correlation was also demonstrated between M1 receptor density and the processing of the amyloid precursor protein, the first event associated with Alzheimer's disease pathogenesis (Rossner et al., 1997). We observed that hypothyroidism resulted in reduced density of α7 and M1 receptors in the hippocampus, an area of the brain responsible for the acquisition and storage of memory. Of the scant information regarding the relationship between the TH and cholinergic receptors, it has been suggested that TH influences the differentiation of cholinergic receptor mRNA, affecting the expression and availability of these receptors in skeletal muscle (Martinou and Merlie, 1991). Accordingly, a considerable reduction in M1 receptor mRNA expression in the hippocampus, related to behavioral deficits in rats, was demonstrated after exposure to the hypothyroid drug propylthiouracil in the perinatal period (Kobayashi et al., 2005).

NeuN is present exclusively the neurons, and its expression can be used as a sensitive, quantitative, and semi-quantitative marker of mature neurons (Yagi et al., 2020). Thus, a decrease in the levels of NeuN is indicative of reduction in the maturation of existing neurons, possibly due to the occurrence of apoptosis before the maturation process or due to the degeneration of already matured neurons (Yadav et al., 2020). In our study, no significant change was observed in the density of NeuN as a consequence of hypothyroidism. However, the possibility of alterations in the neuronal composition upon high exposure to TH deficiency cannot be ruled out and requires further investigation. Other authors demonstrated that hypothyroidism leads to neuronal death in the hippocampus that this mechanism involves oxidative stress, and that activation of the N-methyl-D-aspartate receptor mediates glutamate excitotoxicity (Torres-Manzo et al., 2018). Despite this, the use of resveratrol caused an increase in the density of NeuN, which might indicate a neuroprotective effect of this compound, possibly attributed to its antioxidant activity. Phenolic compounds can reverse the reduction in NeuN expression induced by hypothyroidism, which is likely attributed to their antioxidant activity (Tanaka et al., 2019).

Another molecular marker of tissue conditions in the nervous system is GFAP. GFAP is used to identify astrocytes, which are widely distributed by the central nervous system and are responsible for several processes, including synaptic transmission. TH levels observed in hypothyroidism can affect GFAP expression and, consequently, synapses (Kumar et al., 2018). Herein, we found no significant change in GFAP between the groups. However, reduced levels of GFAP were observed in

an animal model of congenital hypothyroidism (Domingues et al., 2018; Kumar et al., 2018). It is worth noting that the decrease in GFAP density associated with increased AChE activity and oxidative stress demonstrated disrupts of cholinergic transmission (Domingues et al., 2018).

In conclusion, our results indicate that treatment with resveratrol can restore redox homeostasis and cholinergic system functioning in an animal model of hypothyroidism. In view of these findings, and the close relationship between hypothyroidism and cholinergic deficits, resveratrol has potential value in managing brain alterations in patients with hypothyroidism. Moreover, it is relevant to highlight that although researchers have evaluated the action of resveratrol on the cholinergic deficit present in various diseases, to our knowledge, there have been no reports of this polyphenol in a rat model of hypothyroidism.

CRediT authorship contribution statement

Juliane de Souza Cardoso: performed the experimental animal model, the determinations of acetylcholinesterase and oxidative stress parameters, analyzed the results and wrote the manuscript. **Jucimara Baldissarelli:** helped to develop and perform the animal model, edited and corrected the manuscript. **Karine Paula Reichert:** performed the western blot analyzes. **Fernanda Cardoso Teixeira:** helped to perform the determination of oxidative stress parameters. **Mayara Sandrielly Pereira Soares:** helped to perform the acetylcholinesterase activity and to analyze the results. **Maria Rosa Chitolina Schetinger:** provided resources to perform the western blot technique and helped the interpretation of results. **Vera Maria Morsch:** provided resources to perform the western blot technique and assisted the analysis of data. **Antônio Orlando Farias Martins Filho:** performed the measurement of T3 and T4 levels. **Humberto Ribeiro Duarte Junior:** helped to perform the experimental animal model. **Felipe Henrique Ribeiro Coriolano:** helped to perform the experimental animal model. **Roselia Maria Spanevello:** helped to analyze the data and reading the manuscript. **Francieli Moro Stefanello:** provided resources to perform oxidative stress analyzes, assisted the interpretation of data, supervised writing and corrected the manuscript. **Rejane Giacomelli Tavares:** provided resources to perform hormones quantification, designed the study, helped to develop and perform the animal model, supervised the experiments, assisted the interpretation of data and corrected the manuscript.

Declaration of competing interest

None.

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References

Aksenov, M.Y., Markesbery, W.R., 2001. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci. Lett. 302, 141–145.
Ali, S.F., Lebel, C.P., Bondy, S.C., 1992. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. Neurotoxicology 13, 637–648.
Baldissarelli, J., Santi, A., Schmatz, R., Abdalla, F.H., Cardoso, A.M., Martins, C.C., Dias, G.R.M., Calgaroto, N.S., Pelinson, L.P., Reichert, K.P., Loro, V.L., Morsch, V.M. M., Schetinger, M.R.C., 2017. Hypothyroidism enhanced ectonucleotidases and acetylcholinesterase activities in rat synaptosomes can be prevented by the naturally occurring polyphenol quercetin. Cell. Mol. Neurobiol. 37, 53–63.
Baldissarelli, J., Santi, A., Schmatz, R., Zanini, D., Cardoso, A.M., Abadalla, F.H., Thomé, G.R., Murussi, C., Polachini, C.R.N., Delenogare, D.P., Loro, V.L., Morsch, V. M., Schetinger, M.R.C., 2016. Quercetin changes purinergic enzyme activities and oxidative profile in platelets of rats with hypothyroidism. Biomed. Pharmacother. 84, 1849–1857.
Bernal, J., 2002. Action of thyroid hormone in brain. J. Endocrinol. Invest. 25, 268–288.
Biondi, B., Cooper, D., 2019. Thyroid hormone therapy for hypothyroidism. Endocrine 66, 18–26.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.
Campos, C., Casado, A., 2015. Oxidative stress, thyroid dysfunction & down syndrome. Indian J. Med. Res. 142, 113–119.
Cano-Europa, E., Valdivia, V.B., Galindo, G.E.L., Colin, M.F., Reynoso, M.P., Hernandez-Garcia, A., Ortiz-Butron, R., 2010. Methimazole-induced hypothyroidism causes alteration of the REDOX environment, oxidative stress, and hepatic damage; events not caused by hypothyroidism itself. Ann. Hepatol. 9, 80–88.
Cardoso, J.S., Oliveira, P.S., Bona, N.P., Vasconcellos, F.A., Baldissarelli, J., Vizzoto, M., Soares, M.S.P., Ramos, V.P., Spanevello, R.M., Lencina, C.L., Tavares, R.G., Stefanello, F.M., 2018. Antioxidant, antihyperglycemic, and antidyslipidemic effects of Brazilian-native fruit extracts in an animal model of insulin resistance. Redox Rep. 23, 41–46.
Castaldo, L., Narváez, A., Izzo, L., Graziani, G., Gaspari, A., Di Minno, E., Ritieni, A., 2019. Red wine consumption and cardiovascular health. Molecules 24, 3626.
Chaplin, A., Carpéné, C., Mercader, J., 2018. Resveratrol, metabolic syndrome, and gut microbiota. Nutrients 10, 1651.
Colangelo, C., Shichkova, P., Keller, D., Markram, H., Ramaswamy, S., 2019. Cellular, synaptic and network effects of acetylcholine in the neocortex. Front. Neural Circ. 13, 24.
Cuadrado, A., Yubero, C.N., Furneaux, H., Muñoz, A., 2003. Neuronal HuD gene encoding a mRNA stability regulator is transcriptionally repressed by thyroid hormone. J. Neurochem. 86, 763–773.
Dagytė, G., Den Boer, J.A., Trentani, A., 2011. The cholinergic system and depression. Behav. Brain Res. 221, 574–582.
De Assis, P.M., Fávero, A., Menegasso, J.F., Meinel, R.S., Marion, G.M., Nunes, V.S.P., Goliatt, P.V.Z.C., Da Silva, A.D., Dutra, R.C., Raposo, N.R.B., 2020. In silico, in vitro and in vivo studies indicate resveratrol analogue as a potential alternative for neuroinflammatory disorders. Life Sci. 249, 117538.
Dias, G.P., Cocks, G., Bevilaqua, M.C.N., Nardi, A.E., Thuret, S., 2016. Resveratrol: a potential hippocampal plasticity enhancer. Oxidat.Med. Cell. Longev. (2016), 1–14.
Domingues, J.T., Wajima, C.S., Cesconetto, P.A., Parisotto, E.B., Duarte, E.K., Santos, K., Saleh, N., Filippin-Monteiro, F.B., Razzera, G., Silva, F.R.M.B., Pessoa-Pureur, R., Zamoner, A., 2018. Experimentally-induced maternal hypothyroidism alters enzyme activities and the sensorimotor cortex of the offspring rats. Mol. Cell. Endocrinol. 478, 62–76.
Durazzo, A., Lucarini, M., Souto, E.B., Cicala, C., Caiazzo, E., Izzo, A.A., Novellino, E., Santini, A., 2019. Polyphenols: a concise overview on the chemistry, occurrence, and human health. Phytother Res. 33, 1–23.
Ellman, G.L., Courtney, K.D., Andres, V.J., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.
Esterbauer, H., Cheeseman, K.H., 1990. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol. 186, 407–421.
Fukunaga, K., Yabuki, Y., 2018. SAK3-Induced neuroprotection is mediated by nicotinic acetylcholine receptors. In: Akaike, A., Shimohama, S., Misu, Y. (Eds.), Nicotinic Acetylcholine Receptor Signaling in Neuroprotection. Springer, Singapore, pp. 159–171.
Furry, J.D., Mousavi, K., Bolognani, F., Neve, R.L., Parks, R.J., Perrone-Bizzozero, N.I., Jasmin, B.J., 2007. The RNA-binding protein HuD binds acetylcholinesterase mRNA in neurons and regulates its expression after axotomy. J. Neurosci. 27, 665–675.
Gilbert, M.E., Paczkowski, C., 2003. Propylthiouracil (PTU)-induced hypothyroidism in the developing rat impairs synaptic transmission and plasticity in the dentate gyrus of the adult hippocampus. Brain Res. Dev. Brain Res. 145, 19–29.
Gilbert, M.E., 2004. Alterations in synaptic transmission and plasticity in area CA1 of adult hippocampus following developmental hypothyroidism. Brain Res. Dev. Brain Res. 148, 11–18.
Gocmez, S.S., Gacar, N., Utkan, T., Gacar, G., Scarpace, P.J., Tumer, N., 2016. Protective effects of resveratrol on aging-induced cognitive impairment in rats. Neurobiol. Learn. Mem. 131, 131–136.
Guo, H., Cao, H., Cui, X., Zheng, W., Wang, S., Yu, J., 2019. Silymarin's inhibition and treatment effects for alzheimer's disease. Molecules 24, 1748.
Hasegawa, M., Wada, H., 2013. Developmental hypothyroidism disrupts visual signal detection performance in rats. Physiol. Behav. 112, 90–95.
Hu, Y., Xia, Z., Sun, Q., Orsi, A., Rees, D., 2005. A new approach to the pharmacological regulation of memory: sarsasapogenin improves memory by elevating the low muscarinic acetylcholine receptor density in brains of memory-deficit rat models. Brain Res. 1060, 26–39.
Işık, M., Beydemir, Ş., Yılmaz, A., Naldan, M.E., Aslan, H.E., Gülçin, I., 2017. Oxidative stress and mRNA expression of acetylcholinesterase in the leukocytes of ischemic patients. Biomed. Pharmacother. 87, 561–567.
Jena, S., 2015. Effect of persistent and transient hypothyroidism on histoarchitecture and antioxidant defence system in rat brain. Neurol. Sci. 36, 953–959.
Kobayashi, K., Tsuji, R., Yoshioka, T., Kushida, M., Yabushita, S., Sasaki, M., Mino, T., Seki, T., 2005. Effects of hypothyroidism induced by perinatal exposure to PTU on rat behavior and synaptic gene expression. Toxicology 212, 135–147.
Kumar, P., Godbole, N.M., Chaturvedi, C.P., Singh, R.S., George, N., Upadhyay, A., Anjum, B., Godbole, M.M., Sinha, R.A., 2018. Mechanisms involved in epigenetic down- regulation of GFAP under maternal hypothyroidism. Biochem. Biophys. Res. Commun. 502, 375–381.
Leal, J.K.F., Adjobo-Hermans, M.J.W., Brock, R., Bosman, G.J.C.G.M., 2017. Acetylcholinesterase provides new insights into red blood cell ageing in vivo and in vitro. Blood Transfusion 15, 232–238.

Lu, C., Guo, Y., Yan, J., Luo, Z., Luo, H.-B., Yan, M., Huang, L., Li, X., 2013. Design, synthesis, and evaluation of multitarget-directed resveratrol derivatives for the treatment of Alzheimer's disease. J. Med. Chem. 56, 5843–5859.
Ma, X., Sun, Z., Han, X., Li, S., Jiang, X., Chen, S., Zhang, J., Lu, H., 2020. Neuroprotective effect of resveratrol via activation of Sirt1 signaling in a rat model of combined diabetes and Alzheimer's disease. Front. Neurosci. 13, 1–11.
Mancini, A., Di Segni, C., Raimondo, S., Olivieri, G., Silvestrini, A., Meucci, E., Currò, D., 2016. Thyroid hormones, oxidative stress, and inflammation. Mediat. Inflamm. 2016,, 6757154.
Martinez, B., Rodrigues, T.B., Gine, E., Kaninda, J.P., Perez-Castillo, A., Santos, A., 2009. Hypothyroidism decreases the biogenesis in free mitochondria and neuronal oxygen consumption in the cerebral cortex of developing rats. Endocrinology 150, 3953–3959.
Martinou, J.C., Merlie, J.P., 1991. Nerve-dependent modulation of acetylcholine receptor C-subunit gene expression. J. Neurosci. 11, 1291–1299.
Mateo, R.C.I., Hennessey, J., 2019. Thyroxine and treatment of hypothyroidism: seven decades of experience. Endocrine 66, 10–17.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170–3175.
Mokni, M., Elkahoui, S., Limam, F., Amri, M., Aouani, E., 2007. Effect of resveratrol on antioxidant enzyme activities in the brain of healthy rat. Neurochem. Res. 82, 981–987.
Mowla, A., Kalantarhormozi, M.R., Khazraee, S., 2011. Clinical characteristics of patients with major depressive disorder with and without hypothyroidism: a comparative study. J. Psychiatr. Pract. 17, 67–71.
Muramatsu, I., Masuoka, T., Uwada, J., Yoshiki, H., Yazama, T., Lee, K.S., Sada, K., Nishio, M., Ishibashi, T., Taniguchi, T., 2018. A new aspect of cholinergic transmission in the central nervous system. In: Akaike, A., Shimohama, S., Misu, Y. (Eds.), Nicotinic Acetylcholine Receptor Signaling in Neuroprotection. Springer, Singapore, pp. 45–58 (Chapter 3).
Nam, S.M., Kim, J.W., Yoo, D.Y., Jung, H.Y., Chung, J.Y., Kim, D.W., Hwang, I.K., Yoon, Y.S., 2018. Hypothyroidism increases cyclooxygenase-2 levels and pro-inflammatory response and decreases cell proliferation and neuroblast differentiation in the hippocampus. Mol. Med. Rep. 17, 5782–5788.
Nardone, R., Brigo, F., Versace, V., Holler, Y., Tezzon, F., Saltuari, L., Trinka, E., Sebastianelli, L., 2017. Cortical afferent inhibition abnormalities reveal cholinergic dysfunction in Parkinson's disease: a reappraisal. J. Neural. Transm. 124, 1417–1429.
Nelson, D.P., Kiesow, L.A., 1972. Enthalpy of decomposition of hydrogen peroxide by catalase at 25 degrees C (with molar extinction coefficients of H_2O_2 solutions in the UV). Anal. Biochem. 49, 474–478.
Nowsheen, S., Wukovich, R., Aziz, K., Kalogerinis, P.T., Richardson, C.C., Panayiotidis, M.I., Bonner, W.M., Sedelnikova, O.A., Georgakilas, A.G., 2009. Accumulation of oxidatively induced clustered dna lesions in human tumor tissues. Mutation Research, 674, 131–136.
O'Shaughnessy, K.L., Thomas, S.E., Spring, S.R., Ford, J.L., Ford, R.L., Gilbert, M.E., 2019. A transient window of hypothyroidism alters neural progenitor cells and results in abnormal brain development. Sci. Rep. 9, 4662.
Pákáski, M., Kalmán, J., 2008. Interactions between the amyloid and cholinergic mechanisms in Alzheimer's disease. Neurochem. Int. 53, 103–111.
Pan, L.F., Wang, X.B., Xie, S.S., Li, S.Y., Kong, L.Y., 2014. Multitarget-directed resveratrol derivatives: anti-cholinesterases, anti-β-amyloid aggregation and monoamine oxidase inhibition properties against Alzheimer's disease. Med. Chem. Commun. 5, 609–616.
Parodi, O., De Maria, R., Roubina, E., 2007. Redox state, oxidative stress and endothelial dysfunction in heart failure: the puzzle of nitrate-thiol interaction. J. Cardiovasc. Med. (Hagerstown, Md 8, 765–774.
Piccioto, M.R., Higley, M.J., Mineur, Y.S., 2012. Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior. Neuron 76, 116–129.
Poterfield, S.P., 1994. Vulnerability of the developing brain to thyroid abnormalities: environmental insults to the thyroid system. Environ. Health Perspect. 102, 125–130.
Radi, E., Formichi, P., Battisti, C., Federico, A., 2014. Apoptosis and oxidative stress in neurodegenerative diseases. J. Alzheim. Dis. 42 (Suppl. 3). S125-5.
Rebola, N., Pinheiro, P.C., Oliveira, C.R., Malva, J.O., Cunha, R.A., 2003. Subcellular localization of adenosine A(1) receptors in nerve terminals and synapses of the rat hippocampus. Brain Res. 987, 49–58.
Reichert, K.P., Schetinger, M.R.C., Gutierres, J.M., Pelinson, L.P., Stefanello, N., Dalenogare, D.P., Baldissarelli, J., Lopes, T.F., Morsch, V.M., 2018. Lingonberry extract provides neuroprotection by regulating the purinergic system and reducing oxidative stress in diabetic rats. Mol. Nutr. Food Res. 62, e1800050.
Rossner, S., Ueberham, U., Yu, J., Kirazov, L., Schliebs, R., Polo, J.R.P., 1997. In vivo regulation of amyloid precursor protein secretion in rat neocortex by cholinergic activity. Eur. J. Neurosci. 9, 2125–2134.
Salami, M., Bandegi, A.R., Sameni, H.R., Vafaei, A.A., Pakdel, A., 2019. Hippocampal up-regulation of apolipoprotein D in a rat model of maternal Hypo- and hyperthyroidism: implication of oxidative stress. Neurochem. Res. 44, 2190–2201.
Salazar, P., Cisternas, P., Martinez, M., Inestrosa, N.C., 2019. Hypothyroidism and cognitive disorders during development and adulthood: implications in the central nervous system. Mol. Neurobiol. 56, 2952–2963.
Santalucía, T., Palacín, M., Zorzano, A., 2006. T3 strongly regulates GLUT1 and GLUT3 mRNA in cerebral cortex of hypothyroid rat neonates. Mol. Cell. Endocrinol. 251, 9–16.
Schmatz, R., Mazzanti, C.M., Spanevello, R., Stefanello, N., Gutierres, J., Maldonado, P. A., Corrêa, M., da Rosa, C.S., Becker, L., Bagatini, M., Gonçalves, J.F., Jaques, J.S., Schetinger, M.R., Morsch, V.M., 2009. Ectonucleotidase and acetylcholinesterase activities in synaptosomes from the cerebral cortex of streptozotocin-induced diabetic rats and treated with resveratrol. Brain Res. Bull. 80, 371–376.
Seymen, C.M., Gündoğdu, A.C., Bulut, D.I., Demirtaş, C.Y., Elmas, Ç., 2020. Royal jelly increased map-2 expression in hippocampal neurons of hypothyroid rats: an immunohistochemical study. Biotech. Histochem. 95, 46–54.
Serra, M.P., Boi, M., Poddighe, L., Mellis, T., Lai, Y., Carta, G., Quartu, M., 2019. Resveratrol regulates BDNF, trkB, PSA-NCAM, and arc expression in the rat cerebral cortex after bilateral common carotid artery occlusion and reperfusion. Nutrients 11, 1–17.
Singh, S., Sharma, R.K., Malhotra, S., Pothuraju, R., Shandilya, U.K., 2017. Lactobacillus rhamnosus NCDC17 ameliorates type-2 diabetes by improving gut function, oxidative stress and inflammation in high-fat-diet fed and streptozotocintreated rats. Benef. Microbes 8, 243–255.
Stuehr, D.J., Nathan, C.F., 1989. Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. J. Exp. Med. 169, 1543–1555.
Tanaka, T., Masubuchi, Y., Okada, R., Nakajima, K., Nakamura, K., Masuda, S., Nakahara, J., Maronpot, R.R., Toshinori Yoshida, T., Mihoko Koyanagi, M., Hayashi, S.-M., Shibutani, M., 2019. Ameliorating effect of postweaning exposure to antioxidant on disruption of hippocampal neurogenesis induced by developmental hypothyroidism in rats. J. Toxicol. Sci. 44, 357–372.
Tian, X., Liu, Y., Ren, G., Yin, L., Liang, X., Geng, T., Dang, H., An, R., 2016. Resveratrol limits diabetes associated cognitive decline in rats by preventing oxidative stress and inflammation and modulating hippocampal structural synaptic plasticity. Brain Res. 1650, 1–9.
Tiwari, V., Chopra, K., 2013. Resveratrol abrogates alcohol-induced cognitive deficits by attenuating oxidative–nitrosative stress and inflammatory cascade in the adult rat brain. Neurochem. Int. 62, 861–869.
Torres-Manzo, A.P., Franco-Colín, M., Blas-Valdivia, V., Pineda-Reynoso, M., Cano-Europa, E., 2018. Hypothyroidism causes endoplasmic reticulum stress in adult rat Hippocampus: a mechanism associated with hippocampal damage. Oxidat. Med. Cell. Longev. 2018,, 2089404.
Truong, V.L., Jun, M., Jeong, W.S., 2018. Role of resveratrol in regulation of cellular defense systems against oxidative stress. Biofactors 44, 36–49.
Umezu, T., Kita, T., Morita, M., 2019. Hyperactive behavioral phenotypes and an altered brain monoaminergic state in male offspring mice with perinatal hypothyroidism. Toxicol. Rep. 6, 1031–1039.
Ventura, A.L.M., Abreu, P.A., Freitas, R.C.C., Sathler, P.C., Loureiro, N., Castro, H.C., 2010. Sistema colinérgico: revisitando receptores, regulação e a relação com a doença de Alzheimer, esquizofrenia, epilepsia e tabagismo. Archiv. Clin. Psychiatr. 37, 66–72.
Wekking, E.M., Appelhof, B.C., Fliers, E., Schene, A.H., Huyser, J., Tijssen, J.G.P., Wiersinga, W.M., 2005. Cognitive functioning and well-being in euthyroid patients on thyroxine replacement therapy for primary hypothyroidism. Eur. J. Endocrinol. 153, 747–753.
Wu, E.L., Chien, I.C., Lin, C.H., Chou, Y.J., Chou, P., 2013. Increased risk of hypothyroidism and hyperthyroidism in patients with major depressive disorder: a population-based study. J. Psychosom. Res. 74, 233–237.
Xu, Y., Sun, Y., Cheng, J., Xia, Q., Liu, T.-T., Zhu, D.-F., Xu, Q., 2019. Genetic difference of hypothyroidism-induced cognitive dysfunction in C57BL/6j and 129/sv mice. Neurochem. Res. 44, 1999–2006.
Yadav, A., Tandon, A., Seth, B., Goyal, S., Singh, S.J., Tiwari, S.K., Agarwal, S., Nair, S., Chaturvedi, R.K., 2020. Cypermethrin impairs hippocampal neurogenesis and cognitive functions by altering neural fate decisions in the rat brain. Mol. Neurobiol. 2020, 1–18.
Yagi, S., Splinter, J., Tai, D., Wong, S., Wen, Y., Galea, L., 2020. Sex differences in maturation and attrition of adult neurogenesis in the Hippocampus. eNeuro 7, 1–14.
Yong, J.W., Dulcis, D., 2015. Investigating the mechanism(s) underlying switching between states in bipolar disorder. Eur. J. Pharmacol. 759, 151–162.
Zhang, F., Chen, J., Lin, X., Peng, S., Yu, X., Sahn, Z., Teng, W., 2018. Subclinical hypothyroidism in pregnant rats impaired learning and memory of their offspring by promoting the p75NTR signal pathway. Endocrine Connect. 7, 688–697.
Zhang, J.Y., Jiang, H., Gao, W., Wu, J., Peng, K., Shi, Y.F., Zhang, X.J., 2008. The JNK/AP1/ATF2 pathway is involved in H_2O_2-induced acetylcholinesterase expression during apoptosis. Cell. Mol. Life Sci. 65, 1435–1445.
Zhang, W.W., Sun, Q.X., Liu, Y.H., Gao, W., Li, Y.H., Lu, K., Wang, Z., 2011. Chronic administration of Liu Wei Dihuang protects rat's brain against D-galactose-induced impairment of cholinergic system. Acta Physiol. Sin. 63, 245–255.

4.2 Manuscrito 1

Psidium cattleianum fruit extract modulates metabolic, inflammatory and redox alterations in an animal model of type 2 Diabetes Mellitus: comparison with metformin effects

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***Psidium cattleianum* fruit extract modulates metabolic, inflammatory and redox alterations in an animal model of type 2 Diabetes Mellitus: comparison with metformin effects**

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Abstract

Type 2 Diabetes Mellitus (T2DM) is a metabolic disease characterized by hyperglycemia and increased risk of developing nephropathy, neuropathy and cardiovascular diseases. This study sought to investigate the role of *Psidium cattleianum* extract (PCE) (200 mg/kg) in metabolic, inflammatory, oxidative and histological parameters in an animal experimental model of T2DM, induced by a high-fat diet for 3 weeks, followed by a single dose of streptozotocin (STZ) (35 mg/kg). Metformin (Met) (250 mg/kg), positive control, and PCE were administered intragastrically once a day throughout the experiment. Met and PCE were effective in preventing the increase in serum levels of glucose, total cholesterol, triacylglycerols and very low-density lipoprotein (VLDL). Regarding the inflammatory parameters, Met and PCE prevented the increase in the level of interleukin 6 (IL-6) induced by T2DM, but decreased the activity of the paraoxonase-1. On the other hand, Met increased the levels of the anti-inflammatory cytokine, interleukin 10 (IL-10). Met and PCE prevented brain oxidative stress by decreasing the levels of reactive oxygen species, thiobarbituric acid reactive substances and increasing the activity of the antioxidant enzymes superoxide dismutase and catalase. Furthermore, both treatments restored liver and pancreas from marked cellular disorganization, vacuolization and necrosis, with PCE being more effective than Met in recovery of these histological changes. Hence, together the results point to PCE as a promising agent for the prevention of T2DM complications.

Keywords: Diabetes; high-fat diet; streptozotocin; metformin; oxidative stress; phenolic compounds

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorders which is characterized as continuous hyperglycemia and having two main types: type 1 (T1DM) and type 2 (T2DM) (Hamed, 2017). T1DM results from an autoimmune destruction of pancreatic insulin-producing β cells. T2DM is the most prevalent type of DM (90-95% of cases) and it is caused by peripheral insulin resistance (IR). Also, T2DM is commonly associated with overweight and obese individuals and often display an atherogenic dyslipidemia (Hamed, 2017).

Long-term hyperglycemia is related to several complications, such as nephropathy, retinopathy and neuropathy (Faselis et al., 2020). Furthermore, cardiovascular diseases, such as acute myocardial infarction, ischemia and cerebrovascular diseases, are the main cause of death among diabetic patients, and are caused by hyperglycemia, dyslipidemia, increased circulation of free fatty acids (FFA) and oxidative stress (Lehrke and Marx, 2017). The involvement of brain structure and functioning are major causes of neurodegenerative diseases that commonly affect individuals with T2DM and which oxidative stress as one of the major mechanisms involved (Mule and Singh, 2018).

There are different drugs for the treatment of T2DM, ranging from low to high cost drugs; however, the mechanisms of action of these drugs are still discussed and their effectiveness may vary according to the individual's genetics and age, treatment time, among other factors (Gloyn and Drucker, 2018). In addition, side effects may arise, whether mild or severe, such as hypoglycemia, weight gain, seizures, neurological changes, cardiovascular disorders, renal dysfunction and mortality (Gloyn and Drucker, 2018).

Given this scenario, approaches have been developed to discover therapeutic targets that act through different mechanisms, given the complexity of T2DM and its consequences. In this sense, the literature has presented some beneficial effects of the use of natural products as adjuvants in the treatment of T2DM, which go beyond insulin sensitization and hypoglycemic agents, and may act as anti-inflammatory, antioxidant and antidyslipidemic agents (Vivó-Barrachina et al, 2022).

Psidium cattleianum Sabine (*P. cattleianum*) is a specie of succulent fruit, with sub-acid and sweet pulp, popularly known as araçá (Dos Santos Pereira et al., 2018). The *P. cattleianum* fruit is rich in minerals, polysaccharides, carotenoids, phenolic compounds, among other bioactive compounds, which attract the attention of science to the effect of these substances on human health (Dos Santos Pereira et al., 2018). Some beneficial properties have already been described, such as the antimicrobial and antioxidant activity of the leaf extract (Zandoná et al., 2020) and the essential oil (De Souza et al., 2021) of the *P. cattleianum*. The fruit has already been shown to prevent metabolic, biochemical and oxidative changes in animal models of IR (Cardoso et al., 2018) and metabolic syndrome

(Oliveira et al., 2018). In addition, neurochemical and inflammatory changes were prevented by *P. cattleianum* fruit extract (Oliveira et al., 2018, 2020).

Considering that *P. cattleianum* is a native fruit, more studies involving this fruit and the understanding of its action targets in different diseases is necessary. Besides, the search for therapeutic alternatives that prevent the development of T2DM and, consequently, its complications, becomes increasingly relevant since no medication has yet received official sanction as a preventative treatment. Therefore, this study aimed to evaluate the preventive effect of the extract of the native Brazilian fruit *P. cattleianum* (Red type) on metabolic, inflammatory, histopathological and redox parameters in an animal model of T2DM induced by high fat diet and streptozotocin. The effects elicited by fruit extract were compared with metformin, a drug most commonly prescribed to treat T2DM.

2. Materials and Methods

2.1 Extract preparation

Red type native fruits of *P. cattleianum* were obtained from EMBRAPA Clima Temperado (Pelotas, RS, Brazil). After collect, the fruits were immediately stored under refrigeration at - 20ºC and protected from light until use. The extract was made according to Bordignon et al. (2009). Thus, frozen fruits of *P. cattleianum* were sonicated with acidified 70ºGL ethanol (pH 1.0) for 30 min. Then, the *P. cattleianum* extracts (PCE) were filtered, neutralized and evaporated to dryness. The phytochemical characterization of the extracts has been described in previous studies by our research group (Cardoso et al., 2018; Oliveira et al., 2018). LC/PDA/MS/MS analysis demonstrated that cyanidin-3-*O*-glucoside was the only one identified in *P. cattleianum* (Cardoso et al., 2018; Oliveira et al., 2018).

2.2 Animals

Adult male Wistar rats, weighing an average of 350 g, supplied by the Central Animal House of the Federal University of Pelotas were used. The animals were maintained under appropriate conditions, such as a controlled temperature of 22 ± 1°C, a 12 h light/dark cycle, free access to food and water, and maintained in appropriate boxes, with a maximum of 5 animals per box. It should be noted that all procedures adopted with the animals were approved by the Institution's ethics commission (CEEA 5747/2015) and carried out according the “Guide for the Care and Use of Laboratory Animals” (US National Institutes of Health publication no. 85-23, revised 1996).

2.3 Type 2 Diabetes (T2DM) experimental protocol and treatment

The animals were randomly divided into the following groups: Control/Vehicle (CT/V), T2DM/Vehicle (T2DM/V), T2DM/Metformin (T2DM/Met), T2DM/PCE (T2DM/PCE). To induce T2DM, with the exception of the CT/V group, the animals received a high-fat diet (HFD), containing 45% lipids, 35% carbohydrates and 20% protein, for 3 weeks followed by a single intraperitoneal (i.p.) dose of streptozotocin (STZ) (35 mg/kg) dissolved in 0.01 M sodium citrate solution (Singh et al., 2017). During all the experimental period, the animals received vehicle (distilled water), Metformin (Met) (250 mg/kg) (Jiao et al., 2017) or PCE (200 mg/kg) (Cardoso et al., 2018; Oliveira et al., 2018; Oliveira et al., 2020) intragastrically once a day.

2.4 Oral Glucose Tolerance Test (OGTT)

After 72 h of STZ administration and 6 h of fasting, the OGTT was performed. This test consists of monitoring blood glucose using a glucometer (AccuChek Active, Roche Diagnostics®, USA) at times 0, 30, 60 and 120 min after i.p. administration of 50% glucose solution (2 mg/g), from a blood sample collected by a small puncture in the tail of the animals.

2.5 Sample collection

After the experimental protocol, the animals were euthanized and the blood collected and centrifuged at 2500×*g* for 15 min to separate the serum, which was used for biochemical analyses. The cerebral cortex, hippocampus and striatum were separated and used for oxidative stress analyses. The pancreas and visceral adipose tissue were removed and weighed. The liver and pancreas were used for histological analysis.

2.6 Metabolic and serum biochemical parameters

The animals were weighed weekly to monitor weight gain and caloric consumption was evaluated by weighing the food offered and food consumed. Serum measurements of glucose, total cholesterol (TC), triacylglycerol (TG), very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were performed using a colorimetric enzymatic method, using commercial kits supplied by Labtest® (Labtest, MG, Brazil) and BioClin® (BioCLin, MG, Brazil). The fasting triacylglycerol-glucose index (TyG index) was calculated using the formula [Triacylglycerol (mg/dL) x Glucose (mg/dL)/2]. Furthermore, paraoxonase-1 (PON1) activity was evaluated in serum according to the methodology of Browne et al. (2007), considering the rate of phenol formation. For this, the samples were diluted 1:3 in 20 mM Tris/HCl buffer, pH 8.0, containing 1 mM of $CaCl_2$ and then added to the working reagent, which consisted of this buffer and 1 mM phenylacetate as

substrate. Therefore, the reaction was determined after 20 s of retention and the absorbance was measured for 60 s, where one unit of aryl esterase activity was considered equal to 1 mM of phenol formed per minute and expressed in U/mL.

2.7 Oxidative stress parameters

2.7.1 Sample preparation

Cerebral cortex, striatum and hippocampus tissues were homogenized using the sodium phosphate buffer (pH 7.4) containing KCl, centrifuged at 2500×*g* for 10 min at 4°C, and the supernatant was collected for the biochemical analyses. The protein concentration was determined by the methods of Lowry et al. (1951) or Bradford (1976).

2.7.2 Reactive oxygen species (ROS)

ROS levels was measured by the Ali et al. (1992) methodology, in which the emission of fluorescence intensity of 2',7'-dichlorofluorescein (DCF) was recorded at 525 and 488 nm 30 min after the addition of 2',7'-dichloro-dihydrofluorescein diacetate (DCFH-DA) to the medium. Results were expressed as µmol DCF/mg of protein.

2.7.3 Thiobarbituric acid reactive substances (TBARS)

TBARS levels measurement was performed by the method of Ohkawa et al. (1979). In summary, 10% TCA was added to the homogenates and the samples were centrifuged and the supernatant collected. To the supernatant was added 0.67% TBA and then placed in a water bath at 100°C for 30 min. After 10 min in a refrigerator (4°C) the absorbance was analyzed in a spectrophotometer at 535 nm and the results were expressed as nmol TBARS/mg of protein.

2.7.4 Total thiol content

Total thiol content was measured by the method of Aksenov and Markesbery (2001). Generally, PBS buffer containing 1 mM EDTA, pH 7.4, was added to the homogenates and the reaction initiated by the addition of 10 mM DTNB solution. After 60 min of incubation at room temperature and in the dark, absorbance was measured at 412 nm and results were expressed as nmol TNB/mg of protein.

2.7.5 Nitrites

Nitrites measurement was performed using the Griess reaction as described by Sthuer and Nathan (1989). Thus, homogenates are reacted with Griess (1% sulfanilamide, 1% naphthylethylenediamine chloride and 25% H_3PO_4) for 10 min at room temperature. The absorbance was measured at 540 nm and results were expressed as µM nitrite/mg of protein.

2.7.6 Antioxidant enzymes activities

Superoxide dismutase (SOD) activity was evaluated according to the method described by Misra and Fridovich (1972), based on the autoxidation of adrenaline, which is highly dependent on oxygen. One unit of SOD is defined as the amount of SOD required to inhibit 50% of epinephrine autoxidation and the specific activity expressed as units (U)/mg of protein. The catalase (CAT) enzyme activity was determined according to the method of Aebi (1984), where the disappearance of H_2O_2 was continuously monitored in a spectrophotometer at 240 nm for 90 s. One unit of the enzyme is defined as 1 mmol of H_2O_2 consumed per minute and the specific activity reported as U/mg of protein.

2.8 Interleukins

Serum interleukin 6 (IL-6) and interleukin 10 (IL-10) were quantified by ELISA using commercial kits (Sigma-Aldrich, St. Louis, MO, USA).

2.9 Histopathological analysis

Pancreas and liver tissues were fixed in 10% buffered formalin (pH 7.4) and embedded in paraffin. Then, histological sections of 5 µm were performed; slides were prepared and stained with hematoxylin/eosin for histopathological analysis.

2.10 Statistical analysis

Data were analyzed using the software GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). The OGTT was analyzed by repeated measures ANOVA followed by Bonferroni's *post hoc* test. The other parameters were analyzed using one-way ANOVA followed by Tukey *post hoc* test. The $P<0.05$ values were considered as significant differences.

3. Results

3.1 Oral Glucose Tolerance Test (OGTT)

The OGTT was performed to assess the glucose tolerance. Figure 1A shows that T2DM/V group present basal glucose levels higher than other groups (P<0.001) and that after the glucose load, blood glucose remained increased throughout the test, in relation to the other three groups (P<0.001). On the other hand, it is possible to observe that the use of Met and PCE (P<0.001) were effective in preventing impaired ability for glucose disposal when compared to the T2DM/V group (Figure 1).

The area-under-curve (A.U.C) analysis (Figure 1B) also shows the glucose intolerance presented by the T2DM group and the protection against this intolerance by Met and PCE treatment.

3.2 Metabolic and serum biochemical parameters

Table 1 shows data regarding feed consumption and caloric intake estimation and weight gain of the animals during the four weeks of the experiment. It is possible to observe that the average feed consumption in grams remained the same among all groups throughout the experimental period. However, it is estimated that caloric consumption was higher in the first three weeks in the groups that received HFD (T2DM/V (P<0.05), T2DM/Met (P<0.05) and T2DM/PCE (P< 0.01)) compared to the CT/V group, which was expected, since the HFD has more calories than the standard diet consumed by the CT/V group, more specifically, the HFD has 4.59 kcal/g while the standard feed has 2.95 kcal/g of feed (Table 1). In the fourth week, after the administration of STZ, all animals received the standard chow and, thus, the feed consumption, as well as the estimated caloric consumption, was equal between all groups (P>0.05) (Table 1).

Total weight gain was also greater in the groups that received HFD (T2DM/V (P<0.05), T2DM/Met (P<0.05) and T2DM/PCE (P<0.01)) compared to the CT/V group (Table 1). However, after the fourth and last week of the experiment, the total weight gain of these groups became smaller than the CT/V group (Table 1), given the marked weight loss observed after STZ administration (P<0.001) (Figure 2). The visceral adipose tissue relative weight was statistically different only in the T2DM/PCE group, which was higher in relation to the CT/V (P<0.05) and T2DM/V (P<0.05) groups (Table 1). The relative weight of the pancreas showed no significant difference between the groups (P>0.05) (Table 1).

Figure 3 (A-F) presents the results regarding the serum biochemical profile, where they are evidenced significant changes in the T2DM group compared to the CT/V group in relation to blood glucose (P<0.001), TC (P<0.05), TG (P<0.001), VLDL (P<0.001) and HDL (P<0.05) levels. On the other hand, when compared to the T2DM/V group, the groups that

received Met and PCE had lower blood glucose *(P*<0.01), TC (Met *P*<0.01; PCE *P*<0.001), TG (Met: *P*<0.05; PCE: *P*<0.01), and VLDL (Met *P*<0.01; PCE *P*<0.001) levels (Figure 3 (A-D)). Furthermore, PCE treatment caused a decrease in LDL levels compared to the T2DM group (*P*<0.05) (Figure 3E). In HDL levels, treatment with Met or PCE was not effective in preventing the decrease (*P*>0.05) (Figure 3F). The TyG index was increased in the T2DM group compared to the CT/V group (*P*<0.001). On the other hand, treatment with PCE decreased this index in relation to the T2DM group (*P*<0.05), while treatment with Met was not effective in preventing this change (*P*>0.05) (Figure 3G). Regarding the activity of the PON1, it is possible to observe that there was a decrease in the three groups that received HFD and STZ for induction of T2DM (*P*<0.001), and that none of the treatments was effective in preventing this change (Figure 3H).

3.3 Oxidative stress parameters

Table 2 shows important changes observed in brain that are indicative of oxidative stress. It is possible to see that in T2DM group compared to the CT/V group, there was an increase of ROS levels in cortex, striatum and hippocampus (*P*<0.001), as well as TBARS levels in cortex (*P*<0.01), striatum (*P*<0.001) and hippocampus (*P*<0.001). In contrast, the groups that received Met and PCE had lower levels of ROS in the three brain structures (*P*<0.001) and TBARS in cortex (Met *P*<0.05; PCE *P*<0.001), striatum (Met *P*<0.001; PCE *P*<0.001) and hippocampus (Met *P*<0.001; PCE *P*<0.001). Nitrite levels were increased in the T2DM/V group (*P*<0.05) compared to the CT/V group in cortex. On the other hand, the use of PCE prevented this change (*P*<0.05). In striatum and hippocampus, there was no significant change between groups in this parameter (*P*>0.05). Regarding the total thiol content, there was no significant difference between the groups in any of the three evaluated structures (*P*>0.05) (Table 2).

When evaluating the activity of the antioxidant enzymes CAT and SOD, it was possible to observe that the group that received HFD and did not receive treatment caused a decrease in the activity of these enzymes in the cerebral cortex (CAT: *P*<0.01; SOD: *P*<0.01) and striatum (CAT: *P*<0.05; SOD: *P*<0.05). In hippocampus, there was no change in the activity of both enzymes in the T2DM/V group compared to the CT/V group (*P*>0.05). On the other hand, the use of Met prevented the decrease in the activity of these enzymes in cerebral cortex (CAT: *P*<0.01; SOD: *P*<0.05) and striatum (CAT: *P*<0.001; SOD: *P*<0.05). Regarding PCE, it was possible to observe that the use of PCE prevented the decrease in the activity of the CAT enzyme in cerebral cortex (*P*<0.05) and striatum (*P*<0.05) and prevented the decrease in SOD enzyme activity in striatum (*P*<0.05) and also promoted an

increase in SOD enzyme activity in the hippocampus compared to the CT/V and T2DM/V groups (P<0.001) (Table 2).

3.4 Interleukins levels

Figure 4 shows the results for interleukins 6 and 10. A decrease in IL-6 levels can be observed in the T2DM/V group compared to the CT/V group (P<0.001). On the other hand, treatment with Met (P<0.05) and PCE (P<0.05) prevented this decrease. Regarding interleukin 10, it was possible to observe that the use of Met promoted an increase in this interleukin in relation to the CT/V (P<0.001) and T2DM/V (P<0.01) groups (Figure 4).

3.5 Histological analysis

The results about histological analysis of the liver and pancreas are represented in Figure 5 and Figure 6, respectively. The liver of the animals of the T2DM/V group showed marked vacuolization of the hepatocyte cytoplasm and disorganization of the hepatic lobule (Figure 5A), while the CT/V animals showed hepatocytes without vacuolar alterations (Figure 5B). On the other hand, the liver of animals treated with Met showed slight vacuolization of hepatocytes and preserved hepatic lobule (Figure 5C) and the liver of animals in the group that received PCE showed recovery of the hepatic lobular structure and reduction of cytoplasmic vacuolization of hepatocytes (Figure 5D).

The pancreas of animals in the T2DM group showed an important area of necrosis of islets of Langerhans and pancreatic acini (Figure 6B). Meanwhile, the pancreas of animals in the T2DM/Met (Figure 6C) and T2DM/PCE (Figure 6D) groups did not show cellular changes.

4. Discussion

In the present study, we used a high-fat diet (45% fat) followed by a single moderate dose of STZ to induce T2DM, a model widely used in the literature (Srinivasan et al., 2005; Furman, 2015; Singh et al., 2016; Gheibi et al., 2017). The animals that received HFD had an average weight gain of 23% over the three weeks, while the animals that were fed the standard chow gained around 16% in total weight.

Associated with the effects of HFD, the administration of STZ is used to reproduce a T2DM experimental model because it is toxic to pancreatic β cells (Deeds et al., 2011). STZ acts in membrane glucose transporter GLUT-2 by alkylating DNA, inducing the death of pancreatic cells (Deeds et al., 2011). As a consequence, the animals present pancreatic dysfunction and biochemical changes, similarly to those found in humans with T2DM (Gheibi et al., 2017).

Hyperglycemia is the main cause of diabetes complications, associated with morbidity and mortality (Faselis et al., 2020). The measurement of glycemia of the animals that received HFD and a single dose of STZ, that is, the T2DM/V, T2DM/Met and T2DM/PCE groups was 3 times, 1.9 times and 1.8 times higher than that of the CT/V group, respectively. In addition, the use of Met and PCE was able to prevent by 35 and 38%, respectively, the increase in blood glucose.

The TyG index is a marker for the assessment of identifying IR, better than HOMA-IR. Since an increased TG level is an important risk factor, being also strongly associated with cardiometabolic risk, the measurement of TG and glucose and the definition of TyG index represent glycemic control and cardiovascular status simultaneously (Selvi et al., 2021). In this study, there was an increase in the TyG index in the T2DM/V group compared to the control group, which was prevented by PCE. In a study carried out by De Moares et al., (2021), pterostilbene, a polyphenol from the stilbene family already available commercially, was effective in restoring the TyG index, in addition to changes indicative of oxidative stress in animals fed with sucrose-solution. However, the effect *per se* of this polyphenol was not observed in any of the parameters. In addition, pterostilbene did not reverse weight gain, but promoted greater weight gain than CT group, corroborating the effect of PCE on this metabolic parameter. It is important to note that pterostilbene also did not restore the number and size of hepatocytes (De Moraes et al., 2021). Therefore, PCE seems to be more beneficial than compounds already available to the population today, since in addition to the various effects observed in this study, our research group has observed that when administered, including to control animals, the beneficial effects in relation to glycemic parameters, lipids, oxidative stress and liver function remain relevant (Cardoso et al., 2018; Oliveira et al., 2018).

Herein, the use of STZ caused a weight loss of 15.7%, which commonly occurs in other studies that use HFD and different doses of STZ to induce T2DM (Jinshan et al. 2015; Magalhães et al., 2019). The increase in lipolysis results in increased circulation of FFA that contribute to the pathogenesis of T2DM by different mechanisms. In this sense, it is important to mention that the T2DM group presented marked dyslipidemia and treatment with PCE prevented all these changes.

Although there are many data from the literature demonstrating the beneficial action of natural products in the prevention or treatment of metabolic disorders, the mechanisms involved in such effects have not yet been fully clarified. In this context, phenolic compounds most often seem to decrease lipogenesis and de novo fatty acid synthesis, increase lipolysis, attenuate inflammation and oxidative stress (Rodríguez-Pérez et al., 2017). Gómez-Zorita et al. (2017) found that the phenolic compound hesperidin reduced the expression of genes

involved in the three phases of adipocyte synthesis *(c/ebpβ, srebp1c and perilipin*) and reduced TG levels in the cell. On the other hand, the polyphenol apigenin did not affect the expression of these genes and TG levels. In common, the apigenin, hesperidin and kaempferol stimulated the mRNA expression of the lipase enzyme, responsible for fat degradation (Gómez-Zorita et al., 2017). Inactivated lipase is unable to hydrolyze fats, which then pass freely through the feces without being absorbed. In this sense, several natural products, such as extracts and infusions of grape seed (*Vitis vinifera*), white, green and black tea (*Camellia sinensis),* and pomegranate (*Punica granatum*) leaves, have been shown to inhibit the lipase and the fatty acid synthase, and are then considered anti-obesity phytochemicals (Rodríguez-Pérez et al., 2017).

There are two interesting points about PCE that could be mentioned: it promoted the greatest weight gain during the three weeks of HFD and the least weight loss after STZ administration. In addition, the visceral adipose tissue weight was higher compared to the CT group and the T2DM group. As stated earlier, the effects of natural products vary, and in the case of PCE it does not seem to have inhibited adipogenesis, but may have decreased lipolysis. The decrease in lipolysis was possibly responsible for the lower mobilization of FFA and consequent dyslipidemia. The high concentration of FFA in the bloodstream inhibits the IRS/PI3K, preventing the translocation of GLUT-4 to the plasma membrane. Furthermore, FFAs favor the serine phosphorylation of the IRS protein, further impairing insulin signal transduction (Magalhães et al., 2019). In addition to FFA, pro-inflammatory cytokines also promote serine phosphorylation of IRS-1/IRS-2, contributing to abnormal insulin signaling and metabolism (White, 2002), promoting the generation of ROS and the consequent emergence of oxidative stress, both disrupt the functioning of pancreatic β cells and reducing insulin secretion (Agrawal and Kant, 2014).

Oxidative stress is a mechanism directly involved in animal model of several diseases, including IR (Cardoso et al., 2018), metabolic syndrome (Oliveira et al., 2018), neuropsychiatric (Spohr et al., 2019) and neurodegenerative (Teixeira et al., 2020) diseases. In this T2DM model, we found several changes in brain structures indicative of oxidative stress, such as increased levels of ROS and TBARS, evidencing the occurrence of lipid peroxidation, as well as a decrease in the activity of the antioxidant enzymes CAT and SOD. On the other hand, we observed a beneficial effect of PCE in protecting these alterations.

Beyond the oxidative changes, it was possible to observe an increase in the levels of the pro-inflammatory IL-6 and a reduced PON1 activity in diabetic animals; however, PCE and Met prevented the alterations found in the cytokine levels. IL-6 is a cytokine produced by immune system cells, mainly in adipose tissue, which contributes to the onset or worsening of T2DM, inhibiting the expression of insulin receptors, their phosphorylation, as well as IRS-

1 in peripheral and pancreatic tissues (Kojta et al., 2020). Also, the glucotoxicity observed in T2DM may be a key factor inducing IL-6 upregulation and consequent increase of fibrosis IL-6 induced, which is an important pathogenic factor of islet dysfunction (Lu et al, 2021). Furthermore, increased levels of IL-6 decrease adiponectin expression (Kojta et al., 2020), a bioactive molecule secreted by adipocytes, which, among other actions, stimulates IR tyrosine phosphorylation and stimulates the transport and oxidation of fatty acids (Weyer et al., 2001). Thus, decreased levels of adiponectin contribute to important metabolic disorders such as hyperglycemia, dyslipidemia, inflammation, T2DM and atherosclerosis (Weyer et al., 2001; Kojta et al., 2020). On the other hand, IL-10 is an anti-inflammatory cytokine with lower circulating levels in patients T2DM. In contrast, in our study, T2DM animals did not present alteration in IL-10 levels, but Met treatment promoted an increase in the levels of this interleukin, even compared to the CT group. It is well known that Met not only ameliorates chronic inflammation through the improvement of metabolic parameters, but also has a direct anti-inflammatory action (Saisho, 2015). In this context, treatment with Met can reduce expression of IL-1β during long-term exposure to a pro-inflammatory stimulus through a reduction in ROS and an enhanced expression of IL-10 in macrophages (Postler et al., 2021).

PON1 is an enzyme synthesized in the liver, which associated with HDL has the ability to hydrolyze the oxidized LDL molecule, acting as an antioxidant, anti-inflammatory and atheroprotective. Researches indicate a reduction in the expression or activity of the PON1 enzyme, associated with a decrease in HDL levels, in diabetic individuals and, consequently, an increased risk of cardiovascular diseases (El-Said et al., 2015; Namitha et al., 2015). Furthermore, higher intakes of carbohydrates and fat, particularly fat saturated and cholesterol, were associated with lower PON1 activity in subjects with CVD (Longo et al., 2021). A recent study comes as a counterpoint to this finding, demonstrating a positive correlation between diabetes and PON1 enzyme activity, where the enzyme activity was increased in hyperglycemic and hyperinsulinemic diabetic individuals and also showed a significant relationship between the decrease in PON1 activity with increased risk of T2DM (Adiga et al., 2022). Diabetic patients using metformin associated with lifestyle changes, such as limited consumption of simple carbohydrates and fat, greater consumption of fiber and physical activity, had greater PON1 activity associated with decreased of TC and LDL levels, without increasing levels of HDL (Esteghamati et al., 2013). In diabetic animals, treatment with Met (300 mg/kg) for 4 weeks reversed the nearly 25% decrease in PON1 activity, without altering lipid markers (Wójcicka et al., 2016). Furthermore, when administered for 4 weeks in association with curcumin (45 mg/kg), a polyphenol, a natural pigment of turmeric (*Curcuma longa*), Met (250 mg/kg) reversed the decrease in PON1, in

addition to reversing the increase in glucose, TG, TC and markers of oxidative stress. HDL levels were not evaluated in this work (Roxo et al., 2019). In this work, none of the treatments used prevented the decrease in PON1 activity, possibly because they failed to prevent the decrease in HDL and perhaps in association with Met more significant results could be found.

The hyperglycemia in T2DM contributes to oxidative stress and inflammation in different organs and tissues, especially the pancreas. It is important to mention that the pancreas is considered a vulnerable organ to the effects of ROS, as it has a deficient antioxidant defense capacity (Malko and Jiang, 2020). STZ, used for induction of T2DM in this work, is known to destroy pancreatic β cells from the production of ROS; however, other organs can also be affected, such as liver and kidney (Deeds et al., 2011). Given the importance of proper functioning of the pancreas and liver for insulin secretion, adequate glucose and lipid metabolism, synthesis and activity of the PON1 enzyme, among other factors, the aim of this study was to evaluate the histology of these tissues.

In our study, as observed by other authors, the liver and pancreatic tissue of diabetic animals showed important alterations, including necrosis (Abdulmalek et al., 2021). Interestingly, PCE and Met were effective in protecting the tissues against these alterations. According to Boland et al (2017), the dysfunction of pancreatic β cells is the main cause of alteration in glucose metabolism and not the reduction of pancreatic mass. In addition, the reduction or normalization of biochemical parameters promotes a restore of pancreatic β cells, as they decrease the metabolic demand, preserving the secretory function of the pancreas, and thus, contribute to the interruption of the beginning or delay of the progression of T2DM.

Abdulmalek et al. (2021) observed in the liver of animals with induced T2DM, histological changes compatible with hemorrhage, edema and fatty degeneration, in addition to increased expression of IL-1β and TNF-α and markers of oxidative stress in hepatocytes. On the other hand, treatment with Met and curcumin restored the structure of pancreatic tissue, possibly due to the ability of this compound to decrease inflammation and oxidative stress (Abdulmalek et al., 2021). In a study carried out with an animal model of T2DM, the use of quercetin, a flavonoid-like phenolic compound, restored blood glucose, serum insulin levels, IR, oxidative stress markers, antioxidant enzyme activity and important cellular changes in the islets of Langerhans (Li et al., 2020). In this study, despite the changes related to the area and number of islets cells observed in the T2DM group and restored by quercetin, there was no change in the relative weight of the total pancreatic mass (Li et al., 2020).

A mechanism that has recently been pointed out as a possible cause of pancreatic β cell death is ferroptosis. It is characterized by a decrease in mitochondrial volume, reduction or disappearance of mitochondrial cristae and rupture of the mitochondrial outer membrane, and is usually associated with oxidative stress and increased on iron deposition in serum and islet (Li et al., 2020). Iron is a potentially toxic molecule as it can accept and donate electrons. Therefore, the Fenton reaction consists of the oxidation of an iron molecule using H_2O_2, forming the hydroxyl radical ($OH^{\cdot}$), one of the most potent free radicals capable of reacting with various cellular constituents (Imam et al., 2017).

Extracts obtained from fruits rich in phenolic compounds, such as orange, bergamot, grape and chokeberry, or even isolated compounds, such as anthocyanins, curcumin, quercetin and resveratrol, are shown to be effective in protecting different cells from ferroptosis (Imam et al., 2017). Among the mechanisms involved, we highlight iron chelation, decreased iron absorption, prevention of oxidative stress, through the reduction of ROS levels and lipoperoxidation, and increase in the activity and expression of antioxidant enzymes, and also, reduction of inflammation (Imam et al., 2017). In the present study, we observed an antioxidant effect of PCE, through the reduction of ROS and the lipoperoxidation marker and the increase of the activity of the antioxidant enzymes CAT and SOD. It is possible to speculate that the antioxidant effect of PCE could be due to prevention of ferroptosis.

Altogether, our data indicate the *P. cattleianum* fruit extract as an important protector of metabolic, inflammatory, histological and oxidative alterations frequently present in patients with T2DM, as well as of possible complications resulting from this disease. When comparing the effects of ECP with the main drug used to treat T2DM, metformin, we can consider the results presented here even more important, since ECP would be a natural form of treatment, with beneficial actions in different organs and, probably, with fewer adverse effects.

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Conflicts of interest statement

The authors declare no conflicts of interest.

REFERENCES

Abdulmalek, S., Eldala, A., Awad, D. et al., 2021. Ameliorative effect of curcumin and zinc oxide nanoparticles on multiple mechanisms in obese rats with induced type 2 diabetes. Sci Rep, 11: 20677.

Adiga, U., Banawalikar, N., Menambath, D.T. (2022). Association of paraoxonase 1 activity and insulin resistance models in type 2 diabetes mellitus: Cross-sectional study. J Chin Med Assoc, 85: 77-80.

Aebi, H. (1984). Catalase in vitro. Methods Enzymol, 105: 121-126.

Agrawal, N.K., Kant, S., 2014. Targeting inflammation in diabetes: newer therapeutic options. World J Diabetes, 5: 697e710.

Aksenov, M.Y., Markesbery, W.R. (2001). Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci Lett, 302, 141-145.

Ali, S.F., Lebel, C.P., Bondy, S.C. (1992). Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. Neurotoxicology, 13: 637-648.

Boland, B.B., Rhodes, C.J., Grimsby, J.S. (2017). The dynamic plasticity of insulin production in β-cells. Mol Metab, 6: 958-973.

Bordignon, J.R., Francescatto, V., Nienow, A.A. et al. (2009). Influência do pH da solução extrativa no teor de antocianinas em frutos de morango. Ciência e Tecnologia de Alimentos, 29: 183-188.

Bradford M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein– dye binding. Anal Biochem, 72: 248-254.

Browne, R.W., Koury, S.T., Marion, S. et al. (2007). Accuracy and biological variation of human serum paraoxonase 1 activity and polymorphism (Q192R) by kinetic enzyme assay. Clin Chem, 53: 310-317.

Cardoso, J.S., Oliveira, P.S., Bona, N.P. et al. (2018). Antioxidant, antihyperglycemic, and antidyslipidemic effects of Brazilian-native fruit extracts in an animal model of insulin resistance. Redox Report, 23: 41-46.

De Morais, J.M.B., Cruz, E.M.S., Da Rosa, C.V.D. et al. (2021). Pterostilbene influences glycemia and lipidemia and enhances antioxidant status in the liver of rats that consumed sucrose solution. Life Sci, 269: 119048.

De Souza, W.F.C., De Lucena, F.A., De Castro, R.J.S. et al. (2021). Exploiting the chemical composition of essential oils from *Psidium cattleianum* and *Psidium guajava* and its antimicrobial and antioxidant properties. J Food Sci, 86: 4637-4649.

Deeds, M.C., Anderson, J.M., Armstrong, A.S. et al. (2011). Single dose streptozotocin induced diabetes: Considerations for study design in islet transplantation models. Lab Anim, 45: 131-140.

Dos Santos Pereira, E., Vinholes, J., Franzon, R.C. et al. (2018). *Psidium cattleianum* fruits: A review on its composition and bioactivity. Food Chem, 258: 95-103.

El-Said, N.H., Nasr-Allah, M.M., Sadik, N.A. et al. (2015). Paraoxonase-1 activity in type 2 diabetes mellitus with and without nephropathy. Egypt J Intern Med, 27: 63-68.

Esteghamati, A., Eskandari, D., Mirmiranpour, H. et al. (2013). Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. Clin Nutr, 32: 179-85.

Faselis, C., Katsimardou, A., Imprialos, K. et al. (2020). Microvascular Complications of Type 2 Diabetes Mellitus. Curr Vasc Pharmacol, 18: 117-124.

Furman, B.L., 2015. Streptozotocin-induced diabetic models in mice and rats, Curr Protoc Pharmacol, 70: 1–20.

Gheibi, S., Kashfi, K., Ghasemi, A. (2017). A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomed Pharmacother, 95: 605-613.

Gloyn, A.L., Drucker, D.J. (2018). Precision medicine in the management of type 2 diabetes. Lancet Diabetes Endocrinol, 11: 891-900.

Gómez-Zorita, S., Lasa, A., Abendaño, N. et al. (2017). Phenolic compounds apigenin, hesperidin and kaempferol reduce in vitro lipid accumulation in human adipocytes. J Transl Med, 15: 237.

Hamed, S.A. (2017). Brain injury with diabetes mellitus: evidence, mechanisms and treatment implications. Expert Rev Clin Pharmacol, 10: 409-428.

Imam, M.U., Zhang, S., Ma, J. et al. (2017). Antioxidants Mediate Both Iron Homeostasis and Oxidative Stress. *Nutrients, 9:* 671.

Jinshan, J., Zhang, C., Luo, X. et al. (2015). Effect of stay-green wheat, a novel variety of wheat in China, on glucose and on lipid metabolism in high-fat diet induced type 2 diabetic rats. Nutrients, 7: 5143-5155.

Khan, R.M.M., Chua, Z.J.Y., Tan, J.C., et al. (2019). From Pre-Diabetes to Diabetes: Diagnosis, Treatments and Translational Research. Medicina (Kaunas), 55: 546.

Kojta, I., Chacińska, M., Błachnio-Zabielska, A. (2020). Obesity, Bioactive Lipids, and Adipose Tissue Inflammation in Insulin Resistance. Nutrients, 12: 1305.

Lehrke, M., Marx, N. (2017). Diabetes Mellitus and Heart Failure. Am J Med, 130(6S): S40-S50.

Li, D., Jiang, C., Mei, G., et al. (2020). Quercetin Alleviates Ferroptosis of Pancreatic β Cells in Type 2 Diabetes. Nutrients, 12: 2954.

Longo, A., Veiga, G.B., Cousen, M.I.S. et al. (2021). Factors associated to serum paraoxonase 1 activity in patients with cardiovascular disease. Arch Endocrinol Metab,65: 676-683.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. et al. (1951). Protein measurement with the Folin phenol reagent. J Biol Chem, 193: 265-275.

Magalhães, D.A., Kume, W.T., Correia, F.S. et al. (2019). High-fat diet and streptozotocin in the induction of type 2 diabetes mellitus: a new proposal. An Acad Bras Cienc, 91: e20180314.

Malko, P., Jiang, L.H. (2020). TRPM2 channel-mediated cell death: An important mechanism linking oxidative stress-inducing pathological factors to associated pathological conditions. Redox Biol, 37: 101755.

Misra, H.P., Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem, 247: 3170-3175.

Mule, N.K., Singh, J.N. (2018). Diabetes Mellitus to Neurodegenerative Disorders: Is Oxidative Stress Fueling the Flame? CNS Neurol Disord Drug Targets, 17: 644-653.

Namitha, D., Nusrath, A., Rajeshwari, A. et al. (2015). Serum Paraoxonase Levels in Type 2 Diabetes Mellitus Patients: A Case-Control Study. Journal of Medical Sciences and Health, 1: 14-18.

Ohkawa, H., Ohishi, N., Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Ann Rev Med, 95: 351-358.

Oliveira, P.S., Chaves, V.C., Soares, M.S.P. et al. (2018). Southern Brazilian native fruit shows neurochemical, metabolic and behavioral benefits in an animal model of metabolic syndrome. Metab Brain Dis, 33: 1551-1562.

Oliveira, P.S., Soares, M.S.P., Bona, N.P. et al. (2020). Brazilian native fruit extracts act as preventive agents modulating the purinergic and cholinergic signalling in blood cells and serum in a rat model of metabolic syndrome. Arch Physiol Biochem, 26: 1-8.

Postler, T.S., Peng, V., Bhatt, D.M. et al. (2021). Metformin selectively dampens the acute inflammatory response through an AMPK-dependent mechanism. Sci Rep, 11: 18721.

Rodríguez-Pérez, C., Segura-Carretero, A., Del Mar Contreras, M. (2017). Phenolic compounds as natural and multifunctional anti-obesity agents: A review. Crit Rev Food Sci Nutr, 59: 1212-1229.

Roxo, D.F., Arcaro, C.A., Gutierres, V.O. et al. (2019). Curcumin combined with metformin decreases glycemia and dyslipidemia, and increases paraoxonase activity in diabetic rats. Diabetol Metab Syndr, 11: 33.

Saisho, Y. (2015). Metformin and Inflammation: Its Potential Beyond Glucose-lowering Effect. Endocr Metab Immune Disord Drug Targets, 15: 196-205.

Sánchez-García, A., Rodríguez-Gutiérrez, R., Mancillas-Adame, L., et al. (2020). Diagnostic Accuracy of the Triglyceride and Glucose Index for Insulin Resistance: A Systematic Review. Int J Endocrinol, 4678526.

Selvi, N., Nandhini, S., Sakthivadivel, V., Lokesh, S., Srinivasan, A. R., & Sumathi, S. (2021). Association of Triglyceride-Glucose Index (TyG index) with HbA1c and Insulin Resistance in Type 2 Diabetes Mellitus. Maedica, 16(3), 375–381.

Singh, S., Sharma, R.K., Malhotra, S. et al. (2017). *Lactobacillus rhamnosus* NCDC17 ameliorates type-2 diabetes by improving gut function, oxidative stress and inflammation in high-fat-diet fed and streptozotocin treated rats. Benef Microbes, 8: 243-255.

Spohr, L., Soares, M.S.P., Oliveira, P.S., et al. (2019). Combined actions of blueberry extract and lithium on neurochemical changes observed in an experimental model of mania: exploiting possible synergistic effects. Metab Brain Dis, 34: 605-619.

Srinivasan, K., Viswanad, B., Asrat, L. et al. (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res, 4: 313-320.

Stuehr, D.J., Nathan, C.F. (1989). Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. J Exp Med, 169: 1543-1555.

Teixeira, F.C., Gutierres, J.M., Soares, M.S.P. et al. (2020). Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions. Psychopharmacology (Berl), 237: 811-823.

Vivó-Barrachina, L., Rojas-Chacón, M.J., Navarro-Salazar, R. et al. (2022). The Role of Natural Products on Diabetes Mellitus Treatment: A Systematic Review of Randomized Controlled Trials. Pharmaceutics, 14, 101.

Weyer, C., Funahashi, T., Tanaka, S. et al. (2001). Hypoadiponectinemia in obesity and type 2 diabetes: Close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab, 86: 1930–1935.

White, M.F. (2002). IRS proteins and the common pathway to diabetes. Am J Physiol Endocrinol Metab, 283: 413-422.

Wójcicka, G., Jamroz-Wiśniewska, A., Czechowska, G. et al. (2016). The paraoxonase 1 (PON1), platelet-activating factor acetylhydrolase (PAF-AH) and dimethylarginine dimethylaminohydrolase (DDAH) activity in the metformin treated normal and diabetic rats. Eur J Pharmacol, 789: 187-194.

Zandoná, G.P., Bagatini, L., Woloszyn, N. et al. (2020). Extraction and characterization of phytochemical compounds from araçazeiro (*Psidium cattleianum*) leaf: Putative antioxidant and antimicrobial properties. Food Res Int, 137: 1-14.

Table 1. Effect of high-fat diet, STZ administration and treatment with Met and PEC on feed consumption, caloric intake, total weight gain, adipose tissue and pancreas relative weight

	CT/V	T2DM/V	T2DM/Met	T2DM/PCE
Pre STZ or vehicle				
Feed consumption (g/day)	21.68 ± 2.27	17.09 ± 1.44	18.16 ± 0.53	20.69 ± 0.53
Caloric intake (kcal/day)	63.93 ± 6.68	82.52 ± 3.11*	82.82 ± 2.78*	94.31 ± 2.22**
Total weight gain (g)	58.17 ± 4.61	77.67 ± 2.58*	75.17 ± 5.62*	80.86 ± 2.81**
Pos STZ or vehicle				
Feed consumption (g/day)	10.93 ± 0.68	12.78 ± 0.03	14.35 ± 1.59	12.72 ± 0.22
Caloric intake (kcal/day)	32.22 ± 0.09	37.71 ± 0.09	42.32 ±4.70	37.53 ± 0.64
Total weight gain (g)	76.50 ± 4.49	10.57 ± 4.57***	15.63 ± 8.36***	13.89 ± 3.44***
Adipose tissue relative weight	1.48 ± 0.18	1.53 ± 0.07	1.36 ± 0.08	1.83 ± 0.14$^{*\#}$
Pancreas relative weight	0.30 ± 0.0	0.30 ± 0.0	0.30 ± 0.04	0.26 ± 0.02

Data are expressed as mean ± S.E.M. (n =5-9). *** denotes $P < 0.001$, ** denotes $P < 0.01$ and * denotes $P < 0.05$ as compared to the CT/V group. $^{\#\#\#}$ denotes $P < 0.001$, $^{\#\#}$ denotes $P < 0.01$ and $^{\#}$ denotes $P < 0.05$ as compared to the T2DM/V group. One-way ANOVA followed by Tukey *post hoc* test. CT, control; V, vehicle; T2DM, type 2 diabetes mellitus; Met, metformin; PCE, *Psidium cattleianum* extract; STZ, streptozotocin.

Table 2. Effect of treatment with Met and PCE on oxidative stress parameters in cerebral structures of animals submitted to the experimental model of T2DM

	CT/V	T2DM/V	T2DM/Met	T2DM/PCE
Cerebral cortex				
ROS	46.43 ± 8.93	181.5 ± 27.07***	55.48 ± 7.49$^{\#\#\#}$	56.06 ± 4.13$^{\#\#\#}$
TBARS levels	3.34 ± 0.13	5.14 ± 0.39**	3.780 ± 0.13$^{\#}$	2.59 ± 0.34$^{\#\#\#}$
Total thiol content	65.65 ± 9.82	47.33 ± 3.19	72.45 ± 9.47	53.49 ± 3.44
Nitrites levels	24.74 ± 2.98	45.87 ± 7.24*	30.39 ± 3.37	27.85 ± 2.73$^{\#}$
CAT activity	2.01 ± 0.08	1.18 ± 0.11**	1.96 ± 0.18$^{\#\#}$	1.73 ± 0.15$^{\#}$
SOD activity	29.19 ± 0.99	21.73 ± 0.99**	27.30 ± 1.50$^{\#}$	24.98 ± 1.09
Striatum				
ROS	40.32 ± 9.49	95.37 ± 4.92***	25.1 ± 0.80$^{\#\#\#}$	48.1 ± 4.12$^{\#\#\#}$
TBARS	2.73 ± 0.16	4.35 ± 0.33***	2.56 ± 0.18$^{\#\#\#}$	2.44 ± 0.11$^{\#\#\#}$
Total thiol content	57.36 ± 8.61	36.59 ± 4.03	50.57 ± 4.65	51.65 ± 3.37
Nitrite levels	20.14 ± 2.29	18.07 ± 0.31	20.16 ± 1.40	22.09 ± 1.561
CAT activity	1.57 ± 0.19	0.74 ± 0.06*	2.41 ± 0.25$^{\#\#\#}$	1.63 ± 0.11$^{\#}$
SOD activity	33.85 ± 3.24	24.77 ± 1.55*	34.94 ± 2.57$^{\#}$	34.39 ± 1.43$^{\#}$
Hippocampus				
ROS levels	51.59 ± 2.55	225.3 ± 12.80***	66.06 ± 9.82$^{\#\#\#}$	26.63 ± 4.80$^{\#\#\#}$
TBARS levels	2.40 ± 0.12	3.75 ± 0.10***	2.264 ± 0.22$^{\#\#\#}$	2.07 ± 0.13$^{\#\#\#}$
Total thiol content	43.18 ± 4.57	39.05 ± 6.21	41.70 ± 2.36	42.20 ± 2.51
Nitrite levels	18.75 ± 0.99	24.56 ± 3.51	18.27 ± 1.95	18.17 ± 0.83
CAT activity	1.80 ± 0.25	1.57 ± 0.21	1.850 ± 0.15	2.02 ± 0.12
SOD activity	32.17 ± 1.09	29.19 ± 1.88	32.49 ± 1.82	49.30 ± 3.93^{a}

Data are expressed as mean ± S.E.M. (n =4-8). *** denotes $P < 0.001$, ** denotes $P < 0.01$ and * denotes $P < 0.05$ as compared to the CT/V group. $^{\#\#\#}$ denotes $P < 0.001$, $^{\#\#}$ denotes $P < 0.01$ and $^{\#}$ denotes $P < 0.05$ as compared to the T2DM/V group. ^{a} denotes $P < 0.001$ as compared to the CT/V and T2DM/V groups. One-way ANOVA followed by Tukey *post hoc* test. ROS levels are expressed as µmol DCF/mg of protein, nitrite levels are expressed as µM nitrite/mg of protein, thiol content is expressed as nmol TNB/mg of protein, TBARS levels are expressed as nmol TBARS/mg of protein, SOD and CAT activities are expressed as U/mg of protein. CT, control; V, vehicle; T2DM, type 2 diabetes mellitus; Met, metformin; PCE, *Psidium cattleianum* extract; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; CAT, catalase; SOD, superoxide dismutase.

Figure 1

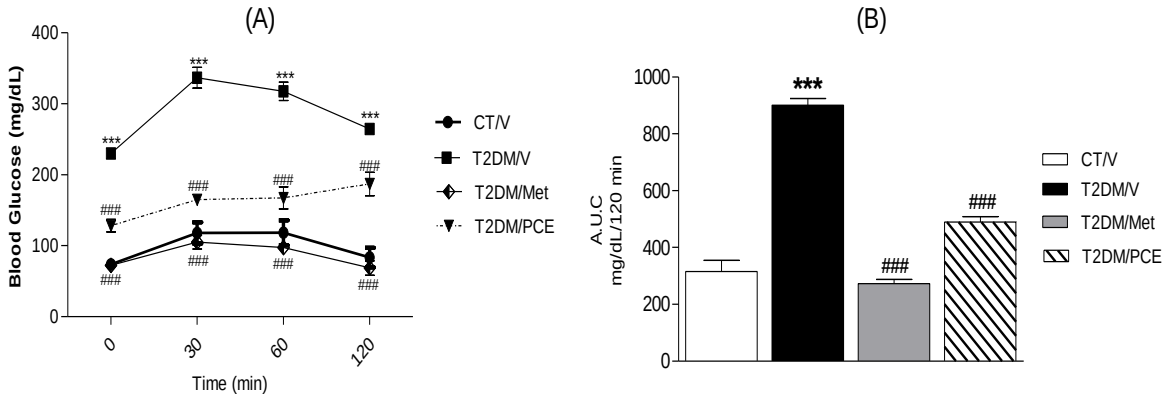
(A)
Blood Glucose (mg/dL)
400
300
200
100
0
0
30
60
120
Time (min)
***
###
CT/V
T2DM/V
T2DM/Met
T2DM/PCE
(B)
A.U.C
mg/dL/120 min
1000
800
600
400
200
0
***
###
CT/V
T2DM/V
T2DM/Met
T2DM/PCE

Figure 2

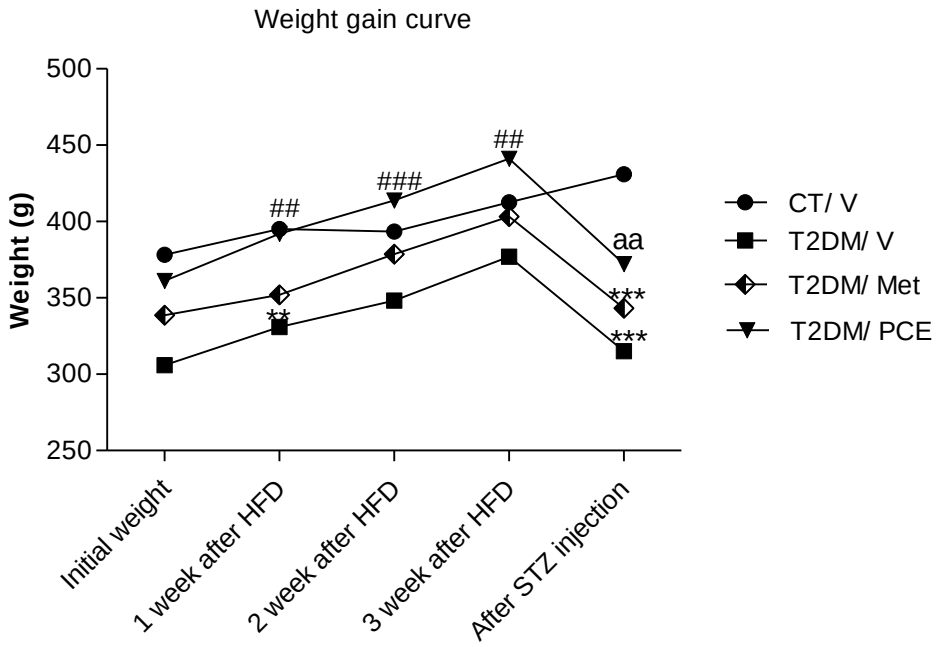
Weight gain curve
Weight (g)
500
450
400
350
300
250
##
###
##
aa
**
***
***
Initial weight
1 week after HFD
2 week after HFD
3 week after HFD
After STZ injection
CT/ V
T2DM/ V
T2DM/ Met
T2DM/ PCE

Figure 3

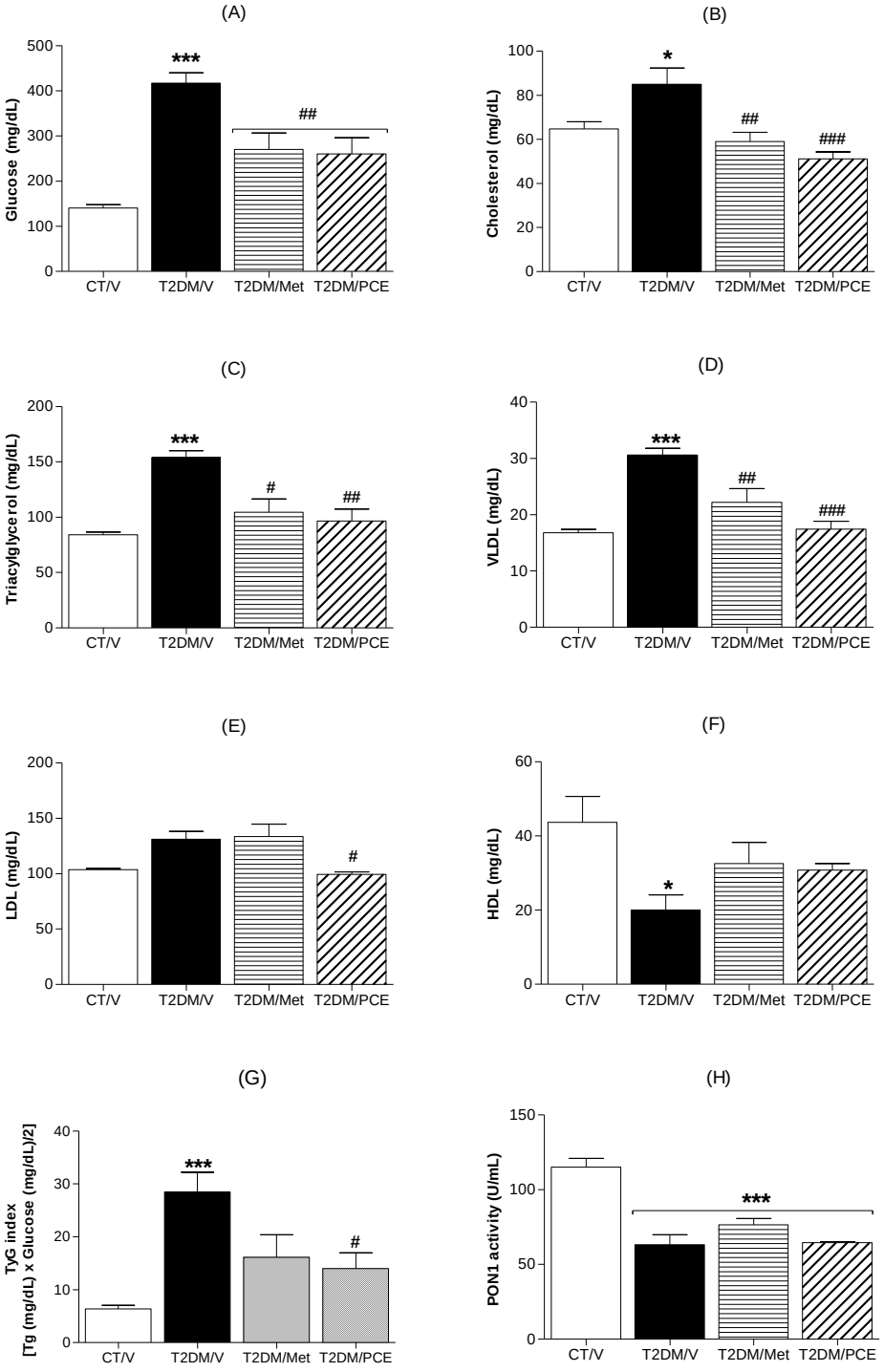
(A)
Glucose (mg/dL)
500
400
300
200
100
0
***
##
CT/V
T2DM/V
T2DM/Met
T2DM/PCE
(B)
Cholesterol (mg/dL)
100
80
60
40
20
0
*
##
###
CT/V
T2DM/V
T2DM/Met
T2DM/PCE
(C)
Triacylglycerol (mg/dL)
200
150
100
50
0
***
#
##
CT/V
T2DM/V
T2DM/Met
T2DM/PCE
(D)
VLDL (mg/dL)
40
30
20
10
0
***
##
###
CT/V
T2DM/V
T2DM/Met
T2DM/PCE
(E)
LDL (mg/dL)
200
150
100
50
0
#
CT/V
T2DM/V
T2DM/Met
T2DM/PCE
(F)
HDL (mg/dL)
60
40
20
0
*
CT/V
T2DM/V
T2DM/Met
T2DM/PCE
(G)
TyG index
[Tg (mg/dL) x Glucose (mg/dL)/2]
40
30
20
10
0
***
#
CT/V
T2DM/V
T2DM/Met
T2DM/PCE
(H)
PON1 activity (U/mL)
150
100
50
0
***
CT/V
T2DM/V
T2DM/Met
T2DM/PCE

Figure 4

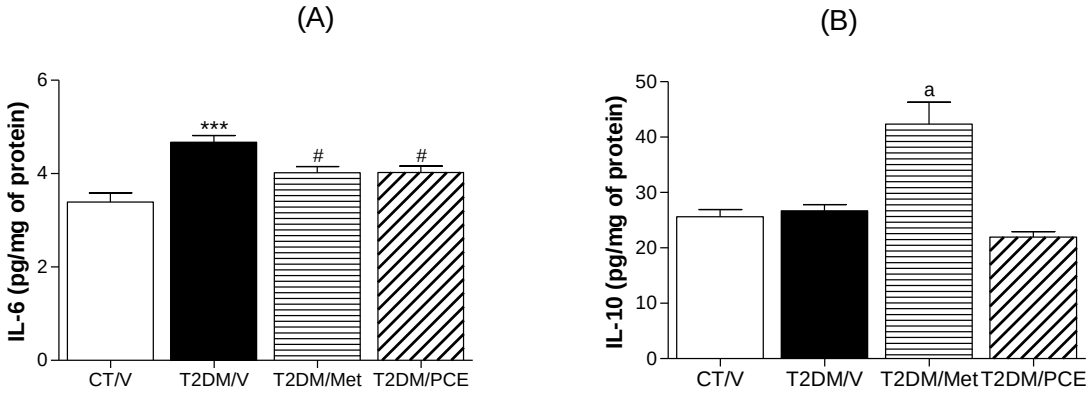
(A)
IL-6 (pg/mg of protein)
6
4
2
0
***
#
#
CT/V
T2DM/V
T2DM/Met
T2DM/PCE
(B)
IL-10 (pg/mg of protein)
50
40
30
20
10
0
a
CT/V
T2DM/V
T2DM/Met
T2DM/PCE

Figure 5

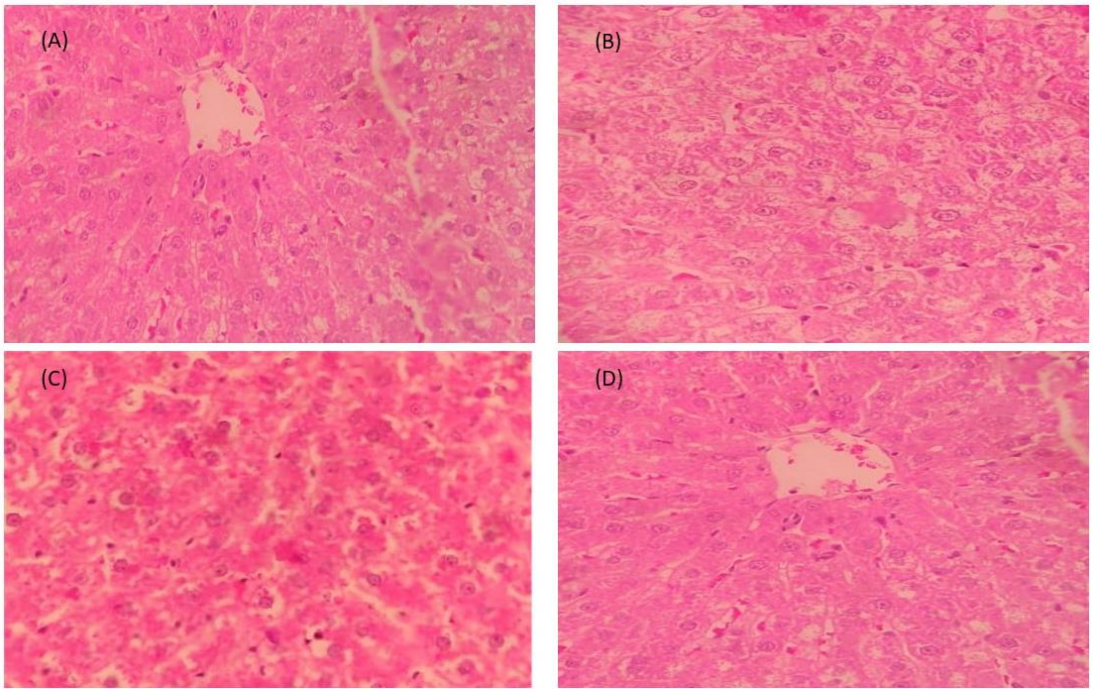

Figure 6

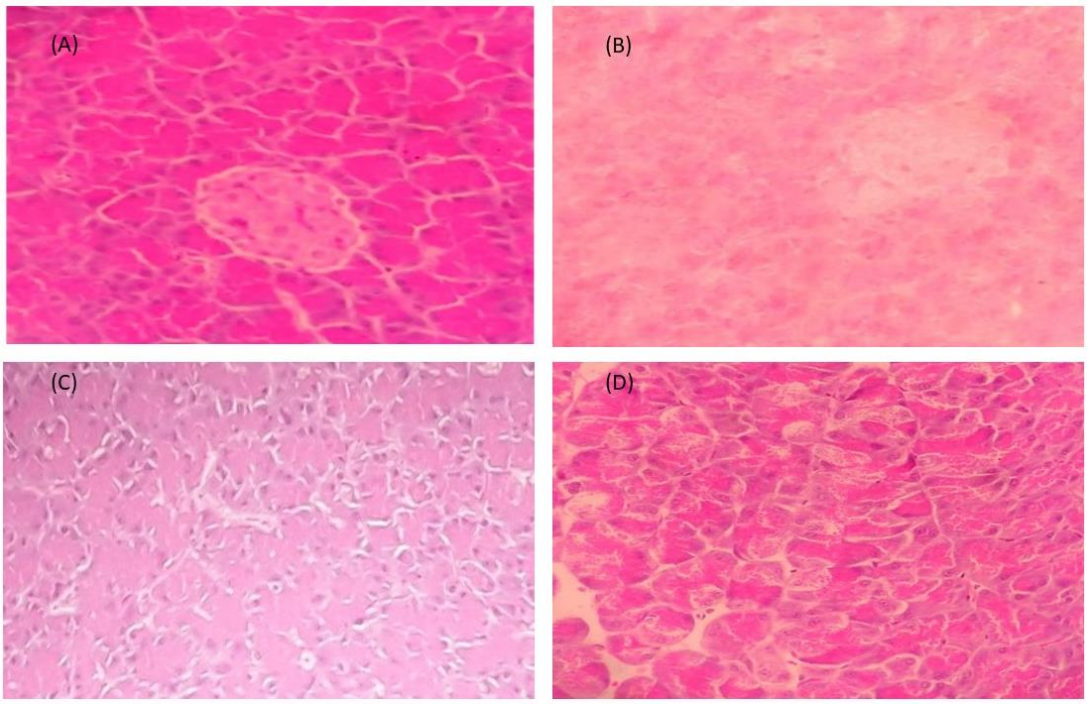

Figure captions

Figure 1: Oral glucose tolerance test demonstrating blood glucose variation before and 30, 60 and 120 minutes after glucose administration (A) and area-under-curve (B). Data are expressed as mean ± S.E.M. (n=4). *** denotes $P < 0.001$ as compared to the CT/V group. ### denotes $P < 0.001$ as compared to the T2DM/V group. Repeated measures ANOVA followed by Bonferroni's *post hoc* test. CT, control; V, vehicle; T2DM, type 2 diabetes mellitus; Met, metformin; PCE, *Psidium cattleianum* extract.

Figure 2: Weight gain over three weeks of HFD consumption or normal diet and weight loss after STZ administration. Data are expressed as mean ± S.E.M. (6-8). *** denotes $P < 0.001$ and ** denotes $P < 0.01$ as compared to the CT/V group. ### denotes $P < 0.001$ and ## denotes $P < 0.01$ as compared to the T2DM/V group. aa denotes $P < 0.01$ as compared to the CT/V and T2DM/V groups. Repeated measures ANOVA followed by Bonferroni's *post hoc* test. CT, control; V, vehicle; T2DM, type 2 diabetes mellitus; Met, metformin; PCE, *Psidium cattleianum* extract.

Figure 3: Effect of treatment with Metformin and *Psidium cattleianum* extract on levels of glucose (A), cholesterol (B), triacylglycerol (C), VLDL (D), LDL (E), HDL, (F) TyG index (G) and on PON1 activity (H) in serum of animals submitted to the experimental model of Type 2 Diabetes Mellitus. Data are expressed as mean ± S.E.M. (4-8). *** denotes $P < 0.001$ and * denotes $P < 0.05$ as compared to the CT/V group. ### denotes $P < 0.001$, ## denotes $P < 0.01$ and # denotes $P < 0.05$ as compared to the T2DM/V group. One-way ANOVA followed by Tukey *post hoc* test. CT, control; V, vehicle; T2DM, type 2 diabetes mellitus; Met, metformin; PCE, *Psidium cattleianum* extract; VLDL, Very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TyG, triacylglycerol-glucose; PON1, paraoxonase-1.

Figure 4: Effect of treatment with Metformin and *Psidium cattleianum* extract on levels of IL-6 (A) and IL-10 (B) in serum of animals submitted to the experimental model of Type 2 Diabetes Mellitus. Data are expressed as mean ± S.E.M. (4-5). *** denotes $P < 0.001$ as compared to the CT/V group. a denotes $P < 0.001$ and 0.01 as compared to the CT/V and T2DM/V group, respectively. One-way ANOVA followed by Tukey *post hoc* test. CT, control; V, vehicle; T2DM, type 2 diabetes mellitus; Met, metformin; PCE, *Psidium cattleianum* extract.

Figure 5: Histopathological characteristics of the liver in type 2 diabetic rats treated with Metformin and *Psidium cattleianum* extract. (A) Control; (B) T2DM, type 2 diabetes mellitus; (C) Metformin; (D) *Psidium cattleianum* extract group. Liver sections were stained with hematoxylin and eosin.

Figure 6: Histopathological characteristics of the pancreas in type 2 diabetic rats treated with Metformin and *Psidium cattleianum* extract. (A) Control; (B) T2DM, type 2 diabetes mellitus; (C) Metformin; (D) *Psidium cattleianum* extract group. Pancreas sections were stained with hematoxylin and eosin.

5 DISCUSSÃO

Hipotireoidismo e DM2 são duas importantes e prevalentes doenças com diversas consequências que aumentam o risco de morte e diminuem a qualidade de vida dos indivíduos. Pacientes portadores de hipotireoidismo possuem níveis diminuídos de HT circulantes e/ou insuficiente resposta do organismo a esses hormônios e, estão sujeitos a importantes consequências, que vão desde fraqueza e distúrbios emocionais à DCV, infertilidade, mau desenvolvimento cerebral fetal e mortalidade (BIONDI et al., 2019). A DM2 é uma doença bem prevalente, que compromete o metabolismo como um todo, resultando em hiperglicemia (OMS, 2016). DCV, como infarto agudo do miocárdio, isquemia e doenças cerebrovasculares, são a principal causa de morte entre os pacientes diabéticos, e são causadas por hiperglicemia, dislipidemia, aumento da circulação de AGL e estresse oxidativo (LEHRKE E MARX, 2017).

Além das doenças acima mencionadas, comumente pacientes diabéticos e hipotireoidianos podem desenvolver um comprometimento cognitivo e até mesmo demência grave (RIEBEN et al., 2016; DE SOUSA et al., 2020). Nessa perspectiva, os dois artigos apresentados nessa tese elucidam alterações presentes em modelo experimental de hipotireoidismo e de DM2. A indução da DM2 com dieta hiperlipídica seguida de uma única dose moderada de STZ para induzir DM2 é amplamente utilizada na literatura (SRINIVASAN et al., 2005; FURMAN, 2015; SINGH et al., 2017; GHEIBI et al., 2017). Os animais desse modelo de DM2 desenvolvem obesidade, RI, destruição parcial das células β-pancreáticas e alterações bioquímicas que naturalmente são encontradas em humanos com a doença (GHEIBI et al., 2017). O envolvimento da estrutura e funcionamento do cérebro são as principais causas de doenças neurodegenerativas que comumente afetam indivíduos com hipotireoidismo e DM2 e que têm o estresse oxidativo como um dos principais mecanismos envolvidos (MULE E SINGH, 2018).

O artigo 1 elucidou as alterações cerebrais presentes no modelo animal de hipotireoidismo, focando no déficit colinérgico e no estresse oxidativo como mecanismos envolvidos na sua fisiopatologia. O hipotireoidismo induzido pelo metimazol causa a diminuição dos HT circulantes, além de estresse oxidativo, indicados pelo aumento dos níveis de ERO e correspondente aumento da peroxidação lipídica, alteração da razão glutationa reduzida e oxidada, bem como

redução da atividade da CAT (CANO-EUROPA et al., 2010). Em nosso trabalho ficou evidente as alterações oxidativas em cérebro e soro. Por outro lado, o uso de resveratrol foi eficaz em proteger contra quase todas as alterações (níveis de ERO, TBARS, conteúdo tiólico total, atividade da enzima CAT em soro e ROS em córtex cerebral e hipocampo). Ainda, é importante ressaltar que até então não haviam relatos desse polifenol em modelo de hipotireoidismo em ratos. Em modelo experimental de hipotireoidismo induzido por metimazol, somente outros compostos foram avaliados e tiveram sua eficácia comprovada em reverter o aumento de TBARS e a redução das atividades de SOD e CAT, como o caso da quercetina, flavonoide avaliado por BALDISSARELLI e colaboradores (2016), tornando estes resultados ainda mais relevantes.

O déficit colinérgico decorre de diversas alterações no sistema colinérgico e, juntamente com neuroinflamação e dano oxidativo fazem parte da fisiopatologia de doenças caracterizadas pelo déficit cognitivo (LAO et al., 2018). Em nosso trabalho, os animais com hipotireoidismo apresentaram aumento, em córtex e hipocampo, da atividade da enzima AChE, responsável pela degradação do neurotransmissor ACh e consequente diminuição da sua disponibilidade na fenda sináptica. Além disso, foi possível observar a diminuição da densidade hipocampal dos receptores de ACh, M1 e α7, que são receptores do tipo muscarinicos e nicotínicos, respectivamente. A diminuição do receptor M1 observada em modelos animais de doenças neurodegenerativas e em ratos idosos tem sido associada a distúrbios de memória e aprendizagem (HU et al., 2005) e pode estar relacionada à menor expressão e disponibilidade desses receptores ocasionada pela deficiência dos HT (MARTINOU E MERLIE, 1991). É importante destacar que o resveratrol ocasionou um aumento na densidade de NeuN, o que pode indicar um efeito neuroprotetor desse composto, possivelmente devido à sua atividade antioxidante. Os compostos fenólicos podem reverter a diminuição da expressão de NeuN causada pelo hipotireoidismo, provavelmente devido à sua atividade antioxidante (TANAKA et al., 2019).

No modelo experimental de DM2 observamos o efeito de *P. cattleianum* em parâmetros bioquímicos, inflamatórios e oxidativos, conforme apresentado no manuscrito 1. De forma significativa, o extrato de *P. cattleianum* preveniu diversas alterações nas estruturas cerebrais indicativas de estresse oxidativo, como aumento dos níveis de ERO e TBARS, evidenciando a ocorrência de peroxidação lipídica,

bem como diminuição da atividade das enzimas antioxidantes CAT e SOD foram observadas. A atividade antioxidante desse fruto já havia sido evidenciada em modelo de RI (CARDOSO et al., 2018) e SM (OLIVEIRA et al., 2018), sendo o manuscrito 1 o primeiro relato do efeito de *P. cattleianum* em modelo animal de DM2.

Além de marcadores de estresse oxidativo em fígado, ABDULMALEK et al. (2012) observaram em fígado de animais com DM2 induzida, alterações histológicas compatíveis com hemorragia, edema e degeneração gordurosa, além de aumento da expressão dos marcadores inflamatórios IL-1β e TNF-α. As citocinas pró-inflamatórias promovem a fosforilação no resíduo serina de IRS-1/IRS-2, impedindo a sinalização da insulina (WHITE, 2002), além de contribuir para o surgimento de distúrbios neurodegenerativos, dada a importância dos componentes da via de sinalização da insulina na proliferação de neurônios e longevidade cerebral (WHITE, 2002). O acúmulo de AGL promove a geração de ERO e o consequente surgimento de estresse oxidativo, que juntamente com a inflamação comprometem o funcionamento das células β-pancreáticas, ou causam sua morte, reduzindo a secreção de insulina (AGRAWAL E KANT, 2014). Por isso, consideramos importante verificar a histologia do fígado e do pâncreas, onde foi possível observar alterações hepáticas como vacuolização e desorganização celular, nos animais que receberam dieta hiperlipídica e STZ. Por outro lado, o tratamento com metformina e extrato de *P. cattleianum* preveniram essas alterações, destacando-se mais o efeito do fruto que do medicamento padrão utilizado como controle positivo. O pâncreas dos animais diabéticos apresentou importante área de necrose das ilhotas de Langerhans e dos ácinos pancreáticos, enquanto o pâncreas dos animais que receberam metformina e extrato não apresentou alterações celulares.

Conforme referido acima, ambos os modelos experimentais foram capazes de mimetizar de forma satisfatória parte da fisiopatologia do hipotireoidismo e da DM2, a partir de alterações metabólicas, bioquímicas, oxidativas, colinérgicas e inflamatórias. Com isso, foi possível avaliarmos o papel dos produtos naturais de interesse frente a essas alterações e assim, compreendermos melhor a importância desses para a ciência.

6 CONCLUSÕES

Essa tese proporcionou uma melhor compreensão dos mecanismos envolvidos no surgimento e agravo de duas relevantes doenças, o hipotireoidismo e a DM2, e principalmente, a elucidação de alguns efeitos benéficos do resveratrol e do extrato de *P. cattleianum* frente as alterações encontradas. Cabe salientar, que estas são as primeiras evidências do resveratrol em modelo animal de hipotireoidimo induzido por metimazol e do fruto de *P. cattleianum* em modelo animal de DM2. Ambos os produtos naturais, mostraram-se efetivos na prevenção de alterações bioquímicas, oxidativas, colinérgicas e histológicas presentes nos animais diabéticos e/ou com hipotireoidismo. Diante dos resultados aqui encontrados, podemos considerar o resveratrol e o fruto de *P. cattleianum* promissores agentes preventivos dessas doenças e de suas consequências.

7 PERSPECTIVAS

Neste trabalho foram apresentados os resultados referentes à avaliação do resveratrol em modelo animal de hipotireoidismo, na forma de um artigo que já foi publicado, e do extrato de *P. cattleianum* em modelo animal de DM2, na forma de um manuscrito que foi submetido à revista científica. Um segundo manuscrito está sendo redigido com resultados complementares do efeito de *P. cattleianum* em modelo de DM2 e, não foi anexado neste trabalho, pois ainda está em fase de preparação. Neste próximo trabalho estamos em busca de uma melhor compreensão dos efeitos do fruto na sinalização da insulina e como isso pode estar relacionado ao efeito anti-hiperglicêmico e antilipidêmico já encontrado e apresentado no manuscrito 1. Para isso, estamos realizando análises moleculares, mais especificamente a expressão dos componentes da via de sinalização da insulina IRS-1, AKT e PI3K. Além disso, a dosagem a insulina em soro será feita, utilizando kit comercial.

Por fim, cabe salientar que um dos objetivos deste segundo manuscrito é compreender a relação da patogênese da DM2 com alterações cerebrais e cognitivas, que parecem ser prevalentes em portadores de DM2. Para isso, a avaliação do sistema colinérgico será realizada a partir da mensuração da atividade das enzimas AChE e BuChE. E ainda, testes comportamentais, como campo aberto e reconhecimento de objetos, foram realizados e estão sendo analisados

estatisticamente, para identificação de possíveis alterações comportamentais indicativas de efeito tipo-depressivo ou ansiedade decorrentes da DM2 e, por outro lado, a prevenção desses distúrbios pelo uso do extrato de *P. cattleianum*.

REFERÊNCIAS

ABDULMALEK, S., ELDALA, A., AWAD, D. et al., 2021. Ameliorative effect of curcumin and zinc oxide nanoparticles on multiple mechanisms in obese rats with induced type 2 diabetes. Sci Rep, 11: 20677.

AEBI, H. Catalase in vitro. Methods in Enzymology, v. 105, p. 121-6, 1984.

AGRAWAL, N.K., KANT, S. Targeting inflammation in diabetes: newer therapeutic options. World J Diabetes, v. 5, p. 697e710, 2014.

AKSENOV, M.Y.; MARKESBERY, W.R. Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci Lett, v. 302, p. 141-5, 2001.

ALI S.F.; LEBEL, C.P.; BONDY, S.C. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. Neurotoxicology, v. 13, p. 637-48, 1992.

ALAPPAT B, ALAPPAT J. Anthocyanin Pigments: Beyond Aesthetics. Molecules, v. 25, p. 5500, 2020.

ALVARENGA, F.Q. et al. In vivo analgesic activity, toxicity and phytochemical screening of the hydroalcoholic extract from the leaves of Psidium cattleianum Sabine. Journal of Ethnopharmacology, v. 150, p. 280-4, 2013.

ANDRISANO, V. et al. A patent review of butyrylcholinesterase inhibitors and reactivators 2010–2017, Expert Opinion on Therapeutic Patents, v. 28, p. 455-65, 2018.

ARAI, I. et al. Improving effects of the extracts from Eugenia uniflora on hyperglycemia and hypertriglyceridemia in mice. Journal of Ethnopharmacology, v. 68, p. 307–14, 1999.

ARAUJO, C. R. M.; SANTOS, V. L. DOS A.; GONSALVES, A. A. Acetilcolinesterase - AChE: Uma Enzima de Interesse Farmacológico Rev. Virtual Quim., v. 8, p. 1818-34, 2016.

BALDISSARELLI, J. et al. Post-thyroidectomy hypothyroidism increases the expression and activity of ectonucleotidases in platelets: Possible involvement of reactive oxygen species. Platelets, v. 29, p. 801-10, 2018.

BALDISSARELLI, J. et al. Quercetin changes purinergic enzyme activities and oxidative profile in platelets of rats with hypothyroidism. Biomed Pharmacother, v. 84, p. 1849-57, 2016.

BEACH, T.G. et al. The cholinergic deficit coincides with Abeta deposition at the earliest histopathologic stages of Alzheimer disease. J Neuropathol Exp Neurol, v. 59, p. 308-13, 2000.

BENSENOR, Isabela. Thyroid disorders in Brazil: the contribution of the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Brazilian Journal of Medical and Biological Research, [s.l.], v. 52, p.1-11, 2019.

BEZERRA, F.J.L et al. Thiobarbituric Acid Reactive Substances as an Index of Lipid Peroxidation in Sevoflurane-Treated Rats. Rev Bras Anestesiol, v. 54, p. 640-9, 2004.

BIONDI, B.; CAPPOLA, A.; COOPER, D. Subclinical Hypothyroidism: A Review. Clinical Review & Education. v. 322, p. 153-60, 2019.

BIONDI, B.; COOPER, D. Thyroid hormone therapy for hypothyroidism. Endocr, v. 66, p. 18-26, 2019.

BODEN, G. et al. Excessive caloric intake acutely causes oxidative stress, GLUT4 carbonylation, and insulin resistance in healthy men. Insulin resistance, v. 7, p. 1-9, 2015.

BORDIGNON, J.R. et al. Influência do pH da solução extrativa no teor de antocianinas em frutos de morango. Ciência e Tecnologia de Alimentos, v. 29, p. 183-8, 2009.

BRADFORD M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein– dye binding. Anal Biochem, v. 72, p. 248-54, 1976.

BRENT, G.A. Mechanisms of thyroid hormones action. The Journal of Clinical Investigation, v. 122, p. 3035-43, 2012.

CAMPOS, C.; CASADO, Á. Oxidative stress, thyroid dysfunction & Down syndrome. Indian Journal of Medical Research, v. 142, p. 113-9, 2015.

CANO-EUROPA, E., VALDIVIA, V.B., GALINDO, G.E.L. et al. Methimazole-induced hypothyroidism causes alteration of the REDOX environment, oxidative stress, and hepatic damage; events not caused by hypothyroidism itself. Ann Hepatol, v. 9, p. 80-88, 2010.

CARDOSO, J.S. et al. Antioxidant, antihyperglycemic, and antidyslipidemic effects of Brazilian-native fruit extracts in an animal model of insulin resistance. Redox Report, v. 23, p. 41-6, 2018.

CASTRO, M.R. et al. Essential oil of Psidium cattleianum leaves: Antioxidant and antifungal activity. Pharmaceutical Biology, v. 53, p. 242-50, 2014.

CARVALHEIRA, J.B.C.; ZECCHIN, H.G.; SAAD, M.J.A. Vias de sinalização da insulina. Arquivo Brasileiro de Endocrinologia e Metabolismo, v. 46, p. 419-425, 2002.

CHAKER, L. et al. Hypothyroidism. Lancet, v. 390, p. 1550-62, 2017.

CHATURVEDI, R. et al. An evaluation of the impact of antidiabetic medication on treatment satisfaction and quality of life in patients of diabetes mellitus. Perspectives in Clinical Research, v. 9, p. 15-22, 2018.

CHENG, Q. et al. Can dipeptidyl peptidase-4 inhibitors treat cognitive disorders? Pharmacology & Therapeutics, p. 1-11, 2020. https://doi.org/10.1016/j.pharmthera.2020.107559

CHIOVATO, L.; MAGRI, F.; CARLÉ, A. Hypothyroidism in Context: Where We've Been and Where We're Going. Advances in therapy, v. 36, p. 47–58, 2019.

DA CUNHA, F.A.B. et al. Cytotoxic and antioxidative potentials of ethanolic extract of Eugenia uniflora L. (Myrtaceae) leaves on human blood cells. Biomedicine & Pharmacotherapy, v. 84, p. 614-21, 2016.

DANGLES, O. Antioxidant Activity of Plant Phenols: Chemical Mechanisms and Biological Significance. Current Organic Chemistry, v. 16, p. 692-714, 2012.

DE SOUSA, R. A. L. et al. An update on potential links between type 2 diabetes mellitus and Alzheimer's disease. Mol Biol Rep, p. 1-10, 2020. https://doi.org/10.1007/s11033-020-05693-z

DELGADO, A.; ISSAQUI, M.; CHAMMEN, N. Analysis of Main and Healthy Phenolic Compounds in Foods. Journal of AOAC International, v. 102, p. 1356-64, 2019.

DELLU, F. et al. Extension of a new two-trial memory task in the rat: influence of environmental context on recognition processes. Neurobiol Learn Mem, v. 67, p. 112–20, 1997.

DEL RIO, D., RODRIGUEZ-MATEOS, A., SPENCER, J.P. et al. A. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxid Redox Signal, v. 18, p. 1818-1892, 2013.

DERE, E.; HUSTON, J. P.; SILVA, M. A. S. Integrated memory for objects, places, and temporal order: Evidence for episodic-like memory in mice. Neurobiology of Learning and Memory, v. 84, p. 214-21, 2005.

DIAS, G.R.M. et al. Diphenyl diselenide diet intake improves spatial learning and memory deficits in hypothyroid female rats. Int J Dev Neurosci, v. 30, p. 83-9, 2012.

DIONÍSIO, T.J.; LOUZADA, J.C.A.; VISCELLI, B.A.; DIONÍSIO, E.J.; MARTUSCELLI, A.M.; BAREL, M. et al. Aerobic Training Prevents Dexamethasone-Induced Peripheral Insulin Resistance. Hormone and Metabolic Research, v. 46, p. 484-489, 2014.

DIRETRIZES DA SOCIEDADE BRASILEIRA DE DIABETES, 2014-2015. Disponível em: https://www.diabetes.org.br/profissionais/images/docs/DIRETRIZES-SBD-2015-2016.pdf Acessado em: 10 de Agosto de 2020.

DIRETRIZES DA SOCIEDADE BRASILEIRA DE DIABETES, 2017-2018. Disponível em: https://www.diabetes.org.br/profissionais/images/2017/diretrizes/diretrizes-sbd-2017-2018.pdf Acessado em: 10 de Agosto de 2020.

DIRETRIZES DA SOCIEDADE BRASILEIRA DE DIABETES, 2018-2019. Disponível em: https://www.diabetes.org.br/profissionais/images/DIRETRIZES-COMPLETA-2019-2020.pdf Acessado em: 10 de Agosto de 2020.

DOMINGUES, J.T. et al. Experimentally-induced maternal hypothyroidism alters enzyme activities and the sensorimotor cortex of the offspring rats. Mol Cell Endocrinol, v. 478, p. 62-76, 2018.

DULAWA, S.C.; JANOWSKY, D.S. Cholinergic Regulation of Mood: From Basic and Clinical Studies to Emerging Therapeutics. Mol Psychiatry, v. 24, p. 694–709, 2019.

DURAZZO, A. et al. Polyphenols: a concise overview on the chemistry, occurrence, and human health. Phytother Res, v. 33, p. 1-23, 2019.

ELLMAN, G.L. et al. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol, v. 7, p. 88-95, 1961.

ERIKSSON, J.W. et al. Sulphonylurea compared to DPP-4 inhibitors in combination with metformin carries increased risk of severe hypoglycemia, cardiovascular events, and allcause mortality. Diabetes Research and Clinical Practice, v. 117, p. 36-47, 2016.

ERSKINE, D. et al. Cholinergic muscarinic M1 and M4 receptors as therapeutic targets for cognitive, behavioural, and psychological symptoms in psychiatric and neurological disorders. Drug Discovery Today, p. 1-8, 2019. https://doi.org/10.1016/j.drudis.2019.08.009

FERREIRA-VIEIRA, T.H. et al. Alzheimer's disease: Targeting the Cholinergic System. Current Neuropharmacology, v. 14, p. 101-15, 2016.

FONSECA, E.A.I. Influência da obesidade e da resistência à insulina sobre o desenvolvimento tumoral: efeito da metformina. 2010, 40 f. Dissertação (Mestrado em Farmacologia) - Programa de Pós-Graduação em Farmacologia. Universidade de São Paulo, São Paulo, 2010.

FRANCIS, P.T.; RAMIREZ, M.J.; LAI, M.K. Neurochemical basis for symptomatic treatment of Alzheimer’s disease. Neuropharmacology, v. 59, p. 221-9, 2010.

FRIEDMAN, J.R. et al. Acetylcholine Signaling System in progression of Lung Cancers. Pharmacol Ther., v. 194, p. 222-54, 2019.

FURMAN, B.L. Streptozotocin-induced diabetic models in mice and rats, Curr Protoc Pharmacol, v. 70, p. 1–20, 2015.

GATTA, V. et al. Possible Correlation between Cholinergic System Alterations and Neuro/Inflammation in Multiple Sclerosis. Biomedicines, v. 8, p. 1-15, 2020.

GAZAL M. et al. Neuroprotective and antioxidant effects of curcumin in a ketamine-induced model of mania in rats. Eur J Pharmacol, v. 5, p. 132–9, 2014.

GE, J.F. et al. Resveratrol Ameliorates the Anxiety- and Depression-Like Behavior of Subclinical Hypothyroidism Rat: Possible Involvement of the HPT Axis, HPA Axis, and Wnt/β-Catenin Pathway. Front Endocrinol, v. 7, p. 1-11, 2016.

GE, J.F. et al. Resveratrol improved the spatial learning and memory in subclinical hypothyroidism rat induced by hemi-thyroid electrocauterization. Endocrine Journal, v. 62, p. 927-38, 2015.

GHANBARI, M.; GHASEMI, A. Maternal hypothyroidism: An overview of current experimental models. Life Sciences, v. 187, p. 1-8, 2017.

GHEIBI, S., KASHFI, K., GHASEMI, A. A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. Biomed Pharmacother, v. 95, p. 605-613, 2017.

GILBERT, M.P.; PRATLEY, R.E. GLP-1 Analogs and DPP-4 Inhibitors in Type 2 Diabetes Therapy: Review of Head-to-Head Clinical Trials. Front Endocrinol, v. 28, p. 1-13, 2020.

GOCMEZ, S.S. et al. Protective effects of resveratrol on aging-induced cognitive impairment in rats. Neurobiol Learn Mem, v.131, p. 131-6, 2016.

HAHN, G.F.; DE OLIVEIRA, J.R.; BOCK, P.M. The role of factor 2 erythroid nuclear factor 2 (Nrf2) in diabetes mellitus. Clin Biomed Res, v. 37, p. 203-13, 2017.

HAJIASGHARZADEH, K. et al. Alpha7 nicotinic acetylcholine receptors in lung inflammation and carcinogenesis: Friends or foes? J Cell Physiol., v. 2019, p. 1-14, 2019.

HAMPEL, H. et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain, v. 141, p. 1917-33, 2018.

HAYES, J.D.; MCLELLAN, L.I. Glutathione and glutathionedependent enzymes represent a co-ordinately regulated defence against oxidative stress, Free Radical Research, v. 31, p. 273-300, 1999.

HE, J., GIUSTI, M.M. Anthocyanins: natural colorants with health-promoting properties. Annu Rev Food Sci Technol, v. 1, p. 163-87, 2020.

HOERMANN, R. et al. Individualised requirements for optimum treatment of hypothyroidism: complex needs, limited options. Drugs in context, v. 8, p. 1-18, 2019.

HOLTHOFF, J.H. et al. Resveratrol, a dietary polyphenolic phytoalexin, is a functional scavenger of peroxynitrite. Biochem Pharmacol, v. 80, p. 1260-5, 2010.

HU, Y., XIA, Z., SUN, Q et al. A new approach to the pharmacological regulation of memory: Sarsasapogenin improves memory by elevating the low muscarinic acetylcholine receptor density in brains of memory-deficit rat models. Brain Research, v. 1060, p. 26-39, 2005.

INTERNATIONAL DIABETES FEDERATION. DIABETES ATLAS Ninth edition 2019. Diponível em: https://www.diabetesatlas.org/upload/resources/2019/IDF_Atlas_9th_Edition_2019.pdf Acessado em: 10 de Agosto de 2020.

ISAH, T. Stress and defense responses in plant secondary metabolites production. Isah Biol Res, v. 52, p. 1-25, 2019.

JABIR, N.R.; KHAN, F.R.; TABREZ, S. Cholinesterase targeting by polyphenols: A therapeutic approach for the treatment of Alzheimer's disease. Neuroscience & therapeutics, v. 24, p. 753-62, 2018.

JAVID, A.Z. et al. Impact of resveratrol supplementation on inflammatory, antioxidant, and periodontal markers in type 2 diabetic patients with chronic periodontitis. Diabetes & Metabolic Syndrome: Clinical Research & Reviews, v. 13, p. 2769-74, 2019.

JENSEN, K.P. et al. The Cholinergic System as a Treatment Target for Opioid Use Disorder. CNS Drugs, v. 32, p. 981-96, 2018.

KANECO, M.; NARUKAWA, M. Meta-analysis of dipeptidyl peptidase-4 inhibitors use and cardiovascular risk in patients with type 2 diabetes mellitus. Diabetes Research and Clinical Practice, v. 116, p. 171-82, 2016.

KARALLIEDDE, J.; GNUDI, L. Diabetes mellitus, a complex and heterogeneous disease, and the role of insulin resistance as a determinant of diabetic kidney disease. Nephrology Dialysis Transplantation, v. 31, p. 206-13, 2014.

KHAN, H. et al. Flavonoids as acetylcholinesterase inhibitors: Current therapeutic standing and future prospects. Biomedicine & Pharmacotherapy, v. 101, p. 860-70, 2018.

KOUKOULI, F.; CHANGEUX, J.P. Do Nicotinic Receptors Modulate High-Order Cognitive Processing? Trends in Neurosciences, v. 43, p. 550-64, 2020.

KRAFT, T.E.; HRESKO, R.C.; HRUZ, P.W. Expression, purification, and functional characterization of the insulin-responsive facilitative glucose transporter GLUT4. Protein Science, v. 24, p. 2008-2019, 2015.

KRISHNASWAMY, A.; COOPER, E. Reactive oxygen species inactivate neuronal nicotinic acetylcholine receptors through a highly conserved cysteine near the intracellular mouth of the channel: implications for diseases that involve oxidative stress. J Physiol, v. 590, p. 39–47, 2012.

LAO, K. et al. Drug Development for Alzheimer's Disease: Review. Journal of Drug Targeting, v. 27, p. 1-30, 2018.

LEE, J.; DURST, R.W.; WROLSTAD, R.E. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants and wines by the pH differential method: Collaborative study. Journal of AOAC Internacional, v. 8, p. 1269-78, 2005.

LEONARD, S.S. et al. Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses. Biochemical and Biophysical Research Communications, v. 309, p. 1017–26, 2003.

LEHRKE, M., Marx, N. Diabetes Mellitus and Heart Failure. American Journal of Medicine, v. 130(6S), p. S40-S50, 2017.

LIONTIRIS, M.; MAZOKOPAKIS, E. A concise review of Hashimoto thyroiditis (HT) and the importance of iodine, selenium, vitamin D and gluten on the autoimmunity and dietary management of HT patients. Points that need more investigation. Hell J Nucl Med, v. 20, p. 51-6, 2017.

LIU, S. et al. Resveratrol oligomers from Paeonia suffruticosa protect mice against cognitive dysfunction by regulating cholinergic, antioxidant and anti-inflammatory pathways. Journal of Ethnopharmacology, v. 260, p. 1-33, 2020.

LOUZADA, R.; CARVALHO, D. Similarities and differences in the peripheral actions of thyroid hormones and their metaboloties. Frontiers in Endocrinology, v. 9, p. 1-14, 2018.

LOWRY, O.H. et al. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, v. 193, p. 265-75, 1951.

LUONGO, Cristina; DENTICE, Mônica; SALVATORE, Domenico. Deiodinases and their intricate role in thyroid hormone homeostasis. Nature Reviews, Endocrinology, v. 15, p. 479-88, 2019.

MARKOWICZ-PIASECKA, M. et al. Metformin – a Future Therapy for Neurodegenerative Diseases. Pharm Res, v. 34, p. 2614-27, 2017.

MAROYI, A. Albizia Adianthifolia: Botany, Medicinal Uses, Phytochemistry, and Pharmacological Properties. The Scientific World Journal, v. 2018, p. 1-8, 2018.

MARTINOU JC, MERLIE JP. Nerve-dependent Modulation of Acetylcholine Receptor C-Subunit Gene Expression. Journal of Neuroscience, v. 11, p. 1291-1299, 1991.

MATEO, R.; HENNESSEY, J. Thyroxine and treatment of hypothyroidism: seven decades of experience. Endocrine, v. 18, p. 1-8, 2019.

MATTIOLI, R., FRANCIOSO, A., MOSCA, L. et al. Anthocyanins: A Comprehensive Review of Their Chemical Properties and Health Effects on Cardiovascular and Neurodegenerative Diseases. Molecules, v. 25, p. 3809, 2020.

MILIAUSKAS, G.; VENSKUTONIS, P.R.; VAN, T.A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry, v. 85, p. 231-7, 2004.

MISRA, H.P.; FRIDOVICH, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. The Journal of Biological Chemistry, v. 247, p. 3170-5, 1972.

MOORADIAN, A.D. Age-Related Resistance to Thyroid Hormone Action. Drugs & Aging, v. 36, p. 1007–14, 2019.

MOURÃO, L.P.S. Análise do gene do substrato do receptor da insulina 1 (irs1) em pessoas com diabetes mellitus tipo 2: identificação de variantes de risco. Tese de doutorado, Universidade do Estado do Amazonas, 2020.

MULE, N.K., SINGH, J.N. Diabetes Mellitus to Neurodegenerative Disorders: Is Oxidative Stress Fueling the Flame? CNS Neurol Disord Drug Targets, v. 17, p. 644-653, 2018.

NJAN, A. A. et al. Neuroprotective and memory-enhancing effects of methanolic leaf extract of Peristrophe bicalyculata in rat model of type 2 diabetes mellitus. Heliyon, v. 6, p. 1-9, 2020.

NOWOTNY, K. et al. Advanced Glycation End Products and Oxidative Stress in Type 2 Diabetes Mellitus. Biomolecules, v. 5, p. 194-222, 2015.

OHKAWA, H.; OHISHI, N.; YAGI, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Annual Review Medicine, v. 95, p. 351-8, 1979.

OLIVEIRA, P.S. et al. Brazilian native fruit extracts act as preventive agents modulating the purinergic and cholinergic signalling in blood cells and serum in a rat model of metabolic syndrome. Archives of Physiology and Biochemistry, v. 26, p. 1-8, 2020.

OLIVEIRA, P.S. et al. Eugenia uniflora fruit (red type) standardized extract: a potential pharmacological tool to diet-induced metabolic syndrome damage management. Biomedicine & Pharmacotherapy, v. 92, p. 935-41, 2017.

OLIVEIRA, P.S. et al. Southern Brazilian native fruit shows neurochemical, metabolic and behavioral benefits in an animal model of metabolic syndrome. Metabolic Brain Disease, v. 11011, p. 1-12, 2018.

ORGANIZAÇÃO MUNDIAL DA SAÚDE. Global report on diabetes, 2016. Disponível em: https://www.who.int/diabetes/global-report/en/ Acessado em: 10 de Fevereiro de 2022.

PATEL, S.S.; RAGHUWANSHI, R.; MASOOD, M.; ACHARYA, A.; JAIN, S.K. Medicinal plants with acetylcholinesterase inhibitory activity. Reviews in the Neurosciences, v. 29, p. 491–529, 2018.

PATEL, T.P. et al. Insulin resistance: an additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes. Heart Failure Reviews, v. 21, p. 11-23, 2016.

PENUMALA, M. et al. Phytochemical profiling and in vitro screening for anticholinesterase, antioxidant, antiglucosidase and neuroprotective effect of three traditional medicinal plants for Alzheimer's Disease and Diabetes Mellitus dual therapy. BMC Complement Altern Med, v. 18, p. 1-13, 2018.

PERRY, E.K. et al. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br Med J, v. 2, p. 1457-9, 1978.

PESSANHA, R.P. Mecanismo de ação do receptor do hormônio tireoideano Beta 1: caracterização molecular e funcional da região de dobradiça e influência do receptor do ácido 9-cis retinóico. 2007. 114 f. Tese (Doutorado em Patologia Molecular)-Universidade de Brasília, Brasília, 2007.

POOLE, L.B. The Basics of Thiols and Cysteines in Redox Biology and Chemistry. Free Radic Biol Med., p. 148-57, 2015. https://doi.org/10.1016/j.freeradbiomed.2014.11.013

POTTER, P.E. et al. Pre- and post-synaptic cortical cholinergic deficits are proportional to amyloid plaque presence and density at preclinical stages of Alzheimer's disease. Acta Neuropathol, v. 122, p. 49-60, 2011.

RANKOVIC, M. et al. Dipeptidyl peptidase-4 inhibitors as new tools for cardioprotection. Heart Failure Reviews, 2020. https://doi.org/10.1007/s10741-020-10005-5

REHMAN, K. et al. Role of Interleukin-6 in Development of Insulin Resistance and Type 2 Diabetes Mellitus. Crit Rev Eukaryot Gene Expr, v. 27, p. 229-236, 2017.

REICHERT, K.P. et al. Lingonberry Extract Provides Neuroprotection by Regulating the Purinergic System and Reducing Oxidative Stress in Diabetic Rats. Mol Nutr Food Res, v. 62, p. 1-7, 2018.

REINISALO, M. et al. Polyphenol stilbenes: molecular mechanisms of defence against oxidative stress and aging-related diseases. Oxidative Medicine and Cellular Longevity, v. 340520, p. 1-24, 2015.

RIBEIRO, A.B. et al. Psidium cattleianum fruit extracts are efficient in vitro scavengers of physiologically relevant reactive oxygen and nitrogen species. Food Chemistry, v. 165, p. 140-8, 2014.

RIVERA, E.J., GOLDIN, A., FULMER, N. et al. Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: Link to brain reductions in acetylcholine. Journal of Alzheimer's Disease, v. 8, p. 247–68, 2005.

RIEBEN, C. et al. Subclinical Thyroid Dysfunction and the Risk of Cognitive Decline: a Meta-Analysis of Prospective Cohort Studies. The Journal of Clinical Endocrinology & Metabolism, v. 101, p. 4945-54, 2016.

RITCHIE, M.; YEAP, B.B. Thyroid hormone: Influences on mood and cognition in adults. Maturitas, v. 81, p. 266-75, 2015.

RIVERA, E.J. et al. Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimer's disease: Link to brain reductions in acetylcholine. Journal of Alzheimer's Disease, v. 8, p. 247–68, 2005.

RONOWSKA, A. et al. The Regulatory Effects of Acetyl-CoA Distribution in the Healthy and diseased brain. Frontiers in Cellular Neuroscience, v. 12, p. 1-20, 2018.

RUBIN, M. A. et al. Intrahippocampal spermidine administration improves inhibitory avoidance performance in rats. Behaviour Pharmacology, v. 11, p. 57-61, 2010.

SALAMA, A.F. et al. Biochemical and histopathological studies of the PTU-induced hypothyroid rat kidney with reference to the ameliorating role of folic acid. Toxicol Ind Health, v. 29, p. 600-8, 2012.

SALAZAR, P. et al. Hypothyroidism and Cognitive Disorders during Development and Adulthood: Implications in the Central Nervous System. Mol Neurobiol, v. 56, p. 2952-63, 2019.

SALTIEL, A.R. Insulin signaling in health and disease. J Clin Invest, v. 131, 2021.

SCHUMACHER, N.S.G. et al. Identification and antioxidant activity of the extracts of Eugenia uniflora leaves. Characterization of the anti-inflammatory properties of aqueous extract on diabetes expression in an experimental model of spontaneous type 1 diabetes (NOD Mice). Antioxidants, v. 4, p. 662-80, 2015.

SHIN, S.S.; DIXON, C.E. Alterations in Cholinergic Pathways and Therapeutic Strategies Targeting Cholinergic System after Traumatic Brain Injury. Journal of Neurotrauma, v. 32, p. 1429–40, 2015.

SILVA, N.M.L. et al. Insulin Resistance as a Shared Pathogenic Mechanism Between Depression and Type 2 Diabetes. Front. Psychiatry, v. 10, p. 1-8, 2019.

SILVERTHORN, Dee Unglaub. Hormônios da Tireoide. In: SILVERTHORN, Dee U. Fisiologia humana: Uma abordagem integrada. 7 ed. São Paulo: Artmed, 2017, 736-740.

SINGH, S., SHARMA, R.K., MALHOTRA, S. et al. *Lactobacillus rhamnosus* NCDC17 ameliorates type-2 diabetes by improving gut function, oxidative stress and inflammation in high-fat-diet fed and streptozotocin treated rats. Benef Microbes, v. 8, p. 243-255, 2017.

SINGLETON, V.L.; ORTHEFER, R.; LAMUELA-RANVENTÓS, R. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. In: Methods Enzymol, (Parker L, ed.), Academic Press, Section III, p. 152-9, 1999.

SNEZHKINA, A. et al. ROS Generation and Antioxidant Defense Systems in Normal and Malignant Cells. Oxidative Medicine and Cellular Longevity, p. 1-17, 2019. https://doi.org/10.1155/2019/6175804

SPOHR, L. et al. Combined actions of blueberry extract and lithium on neurochemical changes observed in an experimental model of mania: exploiting possible synergistic effects. Metabolic Brain Disease, v. 34, p. 605-19, 2018.

SRINIVASAN, K. et al. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacological Research, v. 52, p. 313–20, 2005.

STANCIU, G.D. et al. Alzheimer's Disease Pharmacotherapy in Relation to Cholinergic System Involvement. Biomolecules, v. 10, p. 1-21, 2020.

STUEHR, D.J.; NATHAN, C.F. Nitric oxide. A macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. J Exp Med, v. 169, p. 1543-55, 1989.

SWALLAH, M.S. et al. Antioxidant Potential Overviews of Secondary Metabolites (Polyphenols) in Fruits. International Journal of Food Science, p. 1-8, 2020.

TANAKA, T., MASUBUCHI, Y., OKADA, R. et al. Ameliorating effect of postweaning exposure to antioxidant on disruption of hippocampal neurogenesis induced by developmental hypothyroidism in rats. Journal of Toxicol Science, v. 44, p. 357-372, 2019.

TEIXEIRA, F.C. et al. Inosine protects against impairment of memory induced by experimental model of Alzheimer disease: a nucleoside with multitarget brain actions. Psychopharmacology, v. 237, p. 811–23, 2020.

TENTOLOURIS, A. et al. SGLT2 Inhibitors: A Review of Their Antidiabetic and Cardioprotective Effects. Int. J. Environ. Research Public Health, v. 16, p. 1-27, 2019.

TIAN, X. et al. Resveratrol limits diabetes associated cognitive decline in rats by preventing oxidative stress and inflammation and modulating hippocampal structural synaptic plasticity. Brain Research, v. 2016, p.1-9, 2016.

WHITE, M.F. IRS proteins and the common pathway to diabetes. Am J Physiol Endocrinol Metab, v. 283, p. 413-422, 2002.

WINIARSKA-MIECZAN, A. Protective effect of tea against lead and cadmium-induced oxidative stress—a review. Biometals, v. 31, p. 909–26, 2018.

WORLD HEALTH ORGANIZATION. Classification of diabetes mellitus. Disponível em: https://www.who.int/publications/i/item/classification-of-diabetes-mellitus Acessado em: 10 de Agosto de 2020.

WORLD HEALTH ORGANIZATION. Global report on diabetes, 2016. Disponível em: https://www.who.int/diabetes/global-report/en/ Acessado em: 10 de Agosto de 2020.

WURTZEL, E.T.; KUTCHAN, T.M. Plant metabolism, the diverse chemistry set of the future. Science, v. 353, p. 1232-36, 2016.

ZEIGERER, A.; MCBRAYER, M.K.; MCGRAW, T.E. Insulin stimulation of GLUT4 exocytosis, but not its inhibition of endocytosis, is dependent on RabGAP AS160. Molecular Biology of the Cell, v. 15, p. 4406-4415, 2004.

ZHANG, J. et al. ROS and ROS-Mediated Cellular Signaling. Oxidative Medicine and Cellular Longevity, v. 2016, p. 1-18, 2016.

ZHANG J., LIU F. Tissue-specific insulin signaling in the regulation of metabolism and aging. IUBMB Life. v. 66, p. 485-495, 2014.

Anexo 1- Carta de autorização da Comissão de Ética em Experimentação Animal da Universidade Federal de Pelotas (CEEA-UFPEL) - Protocolo de hipotireoidismo

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **65/2020/CEEA/REITORIA**
PROCESSO Nº 23110.012594/2020-10

Certificado

Certificamos que a proposta intitulada **"AVALIAÇÃO DOS EFEITOS DO RESVERATROL E DE EXTRATOS NATURAIS NOS SISTEMAS PURINÉRGICO E COLINÉRGICO EM SISTEMA NERVOSO CENTRAL E PERIFÉRICO DE RATOS COM HIPOTIREOIDISMO"**, registrada com o nº 23110.012594/2020-10, sob a responsabilidade de **Jucimara Baldissarelli** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de **10/06/2020**.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	01/08/2020 a 01/058/2023
Espécie/linhagem/raça	*Rattus norvegicus*/Wistar
N° de animais	190
Idade	60 dias
Sexo	Machos
Origem	Biotério Central - UFPel

Anexo 2- Carta de autorização da Comissão de Ética em Experimentação Animal da Universidade Federal de Pelotas (CEEA-UFPEL) - Protocolo de DM2

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **90/2018/CEEA/REITORIA**
PROCESSO Nº 23110.031567/2018-13

Pelotas, 15 de agosto de 2018

Certificado

Certificamos que a **solicitação de adendo** proposta intitulada **"Avaliação das atividades antioxidante, hipocolesterolêmica e/ou hipoglicemiante de produtos naturais"** processo com cadastro CEEA 5747-2015, de responsabilidade de **Francieli Moro Stefanello** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua complementação pela Comissão de Ética em Experimentação Animal, em reunião de 13/08/2018.

Prorrogação de prazo de execução até 31 de dezembro de 2020

M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da CEEA

Documento assinado eletronicamente por **ANELIZE DE OLIVEIRA CAMPELLO FELIX**, **Médico Veterinário**, em 15/08/2018, às 11:02, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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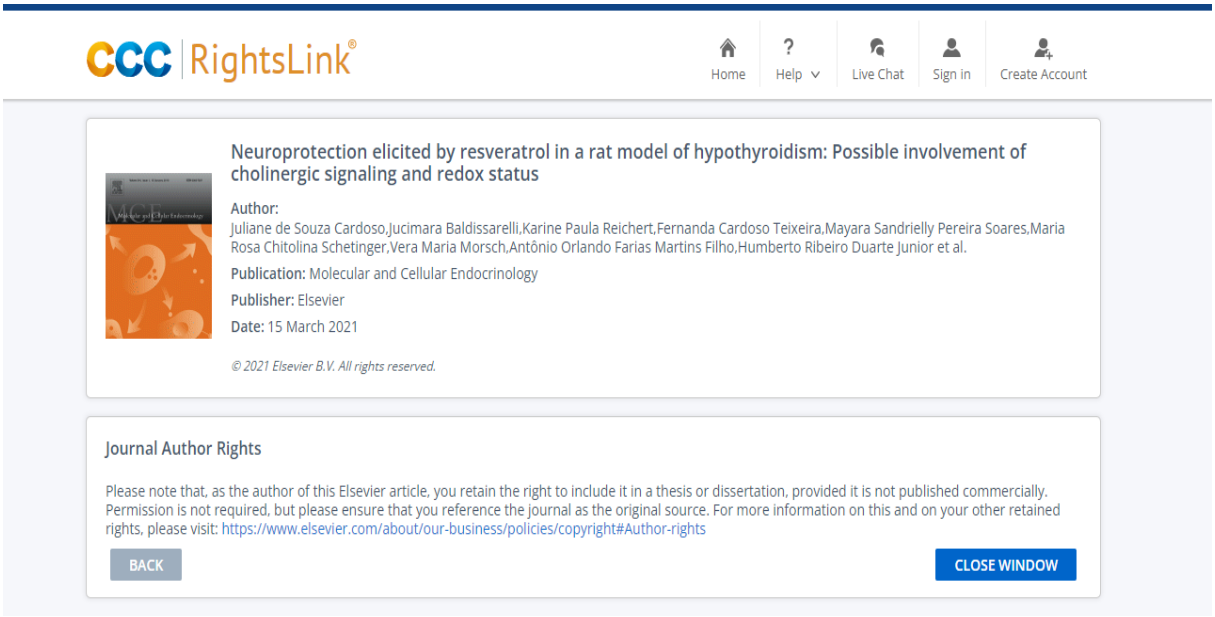
Referência: Processo nº 23110.031567/2018-13 SEI nº 0242484

Anexo 3- Permissão da Revista *Molecular and Cellular Endocrinology* para inclusão do Artigo 1 na Tese



Anexo 4- Comprovante de submissão do Manuscrito 1

Submission Confirmation

Print

Thank you for your submission

Submitted to
Free Radical Research

Manuscript ID
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Title
Psidium cattleianum fruit extract prevents metabolic, redox and histopathological alterations in an animal model of type 2 Diabetes Mellitus: comparison with metformin effects

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