UNIVERSIDADE FEDERAL DO PARANÁ

ANA PAULA FARIAS WALTRICK

INVESTIGAÇÃO DO POTENCIAL TERAPÊUTICO DO MEDIADOR LIPÍDICO PRÓ-RESOLUÇÃO DA INFLAMAÇÃO PROTECTINA DX SOBRE AS RESPOSTAS COMPORTAMENTAIS DE DIFERENTES COMORBIDADES ASSOCIADAS AO DIABETES *MELLITUS* TIPO 1 INDUZIDO EXPERIMENTALMENTE EM RATOS

CURITIBA

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Tese apresentada ao curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Farmacologia.

Orientadora: Profa. Dra. Janaina Menezes Zanoveli

Coorientadora: Profa. Dra. Joice Maria da Cunha

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ATA Nº332

ATA DE SESSÃO PÚBLICA DE DEFESA DE DOUTORADO PARA A OBTENÇÃO DO GRAU DE DOUTORA EM FARMACOLOGIA

No dia vinte de outubro de dois mil e vinte e tres às 09:00 horas, na sala auditório, Auditório da farmacologia, foram instaladas as atividades pertinentes ao rito de defesa de tese da doutoranda **ANA PAULA FARIAS WALTRICK**, intitulada**: Investigação do potencial terapêutico do mediador lipídico pró-resolução da inflamação Protectina DX sobre as respostas comportamentais de diferentes comorbidades associadas ao diabetes *mellitus* tipo 1 induzido experimentalmente em ratos**, sob orientação da Profa. Dra. JANAÍNA MENEZES ZANOVELI. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: JANAÍNA MENEZES ZANOVELI (UNIVERSIDADE FEDERAL DO PARANÁ), VANESSA DE PAULA SOARES RACHETTI (UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE), LUIZ KAE SALES KANAZAWA (55001467), FRANCISLAINE APARECIDA DOS REIS LIVERO (UNIVERSIDADE FEDERAL DO PARANÁ). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de doutora está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, JANAÍNA MENEZES ZANOVELI, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 20 de Outubro de 2023.

Assinatura Eletrônica
01/11/2023 16:37:14.0
JANAÍNA MENEZES ZANOVELI
Presidente da Banca Examinadora

Assinatura Eletrônica
27/10/2023 15:00:32.0
VANESSA DE PAULA SOARES RACHETTI
Avaliador Externo (UNIVERSIDADE FEDERAL DO RIO GRANDE DO NORTE)

Assinatura Eletrônica
24/10/2023 17:10:58.0
LUIZ KAE SALES KANAZAWA
Avaliador Externo (55001467)

Assinatura Eletrônica
25/10/2023 11:43:10.0
FRANCISLAINE APARECIDA DOS REIS LIVERO
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **ANA PAULA FARIAS WALTRICK** intitulada: **Investigação do potencial terapêutico do mediador lipídico pró-resolução da inflamação Protectina DX sobre as respostas comportamentais de diferentes comorbidades associadas ao diabetes *mellitus* tipo 1 induzido experimentalmente em ratos**, sob orientação da Profa. Dra. JANAÍNA MENEZES ZANOVELI, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 20 de Outubro de 2023.

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NOTA EXPLICATIVA

Esta tese é apresentada em formato alternativo (artigo científico) – de acordo com as normas do Programa de Pós-Graduação em Farmacologia da Universidade Federal do Paraná. Neste formato, o texto apresenta uma introdução estendida e objetivos do trabalho (em português), e o artigo científico do estudo realizado (em inglês) abordando experimentos realizados, resultados e discussão. Por fim, considerações finais (em português). O artigo foi formatado conforme as normas de periódicos internacionais.

Este artigo está relacionado diretamente com as atividades da tese. O primeiro artigo obrigatório do doutorado é relacionado com outro projeto de pesquisa e pode ser encontrado a partir da seguinte citação:

Waltrick APF, da Silva ACF, de Mattos BA, et al. Long-term treatment with roflumilast improves learning of fear extinction memory and anxiety-like response in a type-1 diabetes *mellitus* animal model. Behav Brain Res. 2023;439:114217. doi:10.1016/j.bbr.2022.114217

Dedico este trabalho à universidade pública, gratuita e de qualidade.

À Universidade Federal do Paraná, que foi minha casa pelos últimos 12 anos.

AGRADECIMENTOS

Além desses agradecimentos representarem o fim do doutorado, eles representam o final de um ciclo tão importante quanto: minha passagem como aluna pela Universidade Federal do Paraná. Trilho este caminho desde 2011, e durante esses quase 13 anos de UFPR, passei pela graduação (bacharelado e licenciatura), mestrado e doutorado. Tudo isso não seria possível sem os mencionados aqui, cada um com sua importância e lugar especial em minha vida!

Agradeço à minha família, principalmente meus pais Sandra e Luciano, e irmãos Carol e José, por sempre me apoiarem nas minhas decisões e por me ajudarem a chegar aonde eu quero. Vocês têm uma participação IMENSA em todas as minhas conquistas e eu não poderia ser mais grata.

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A todos os membros do departamento de Farmacologia da UFPR, alunos, professores e funcionários, por facilitarem esse processo que é a pós-graduação em um ambiente tão amigável e acolhedor sempre. Em especial aos amigos que fiz nesse caminho. À professora Alexandra Acco por ter aberto as portas do seu laboratório para que eu pudesse realizar as análises de estresse oxidativo, e ao Kauê e à Débora que me ajudaram com esses experimentos, além de serem amigos incríveis.

Agradeço ao Lab Jana, em todas as suas versões, e pelo qual eu fiz parte nos últimos 7 anos! E principalmente ao meu trio preferido, Alvaro e Yane, meus parceiros de ciência e amizade mais sincera que a pós proporcionou. Ao Lab Dor, pelo espaço físico, pelos amigos que fiz, pelas portas abertas sempre. Principalmente à professora Joice, minha coorientadora e amiga, por todos os conselhos, parcerias, amizade.

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A todos que, por algum motivo, fazem parte da minha caminhada, o meu mais sincero “muito obrigada”! Nós chegamos lá!

“Não sou esperançoso por pura teimosia, mas por imperativo existencial e histórico.

Não quero dizer, porém, que, porque esperançoso, atribuo à minha esperança o poder de transformar a realidade e, assim, convencido, parto para o embate sem levar em consideração os dados concretos, materiais, afirmando que minha esperança basta. Minha esperança é necessária, mas não é suficiente. Ela, só, não ganha a luta, mas sem ela a luta fraqueja e titubeia.”

Paulo Freire, 1992.

RESUMO

A Protectina DX (PDX), um mediador lipídico pró-resolução da inflamação, possui potencial terapêutico em diversas condições médicas devido às suas propriedades anti-inflamatórias e antioxidantes. Tendo em vista doenças com características inflamatórias e oxidativas, como o diabetes *mellitus*, torna-se necessário explorar o potencial terapêutico da PDX no tratamento do diabetes *mellitus* tipo 1 (DMT1) e as comorbidades associadas, incluindo dor neuropática diabética, depressão e ansiedade. Estas condições compartilham mecanismos fisiopatológicos comuns, como o aumento na produção de espécies reativas de oxigênio. No presente estudo investigamos, em ratos com DMT1 induzido, a eficácia da PDX no alívio da dor neuropática (alodinia mecânica; experimento 1), comportamentos do tipo ansiedade e do tipo depressivo (experimento 2). Além disso, estudamos se o tratamento com a PDX induziria efeitos antioxidantes no hipocampo, no córtex pré-frontal e no plasma sanguíneo (experimento 3). Após 2 semanas de indução de DMT1 com estreptozotocina (60 mg/kg) em ratos *Wistar* (110 animais), PDX (1, 3 e 10 ng/animal; injeção ip de 200 µl/animal) foi administrada continuamente nos dias 14, 65, 18, 21, 24 e 27 após a indução do diabetes. Todas os experimentos foram realizados em grupos independentes de ratos. A PDX aumentou consistentemente o limiar mecânico ao longo do estudo em todas as doses, indicativo de efeito antinociceptivo. Para avaliação das respostas de ansiedade, os animais foram submetidos aos testes de labirinto em cruz elevado (dia 26) e campo aberto (dia 28), enquanto para comportamento tipo depressivo, os animais foram submetidos ao teste de natação forçada modificado (sessão teste no dia 28, com pré-teste no dia 27). Todas as doses da PDX preveniram eficazmente os comportamentos de tipo depressivo e de ansiedade, em comparação com ratos com DMT1 tratados com veículo salina. O tratamento com a PDX protegeu significativamente contra o aumento dos parâmetros de estresse oxidativo no hipocampo, córtex pré-frontal e plasma sanguíneo. Os animais tratados melhoraram os parâmetros diabéticos, apresentando ganho de peso e níveis reduzidos de glicemia em ratos com DMT1. Esses achados sugerem que a PDX apresenta ação do tipo antidepressiva e ansiolítica em animais com DMT1 induzido, além de melhorar parâmetros relacionados ao quadro diabético. Vale ressaltar que a PDX também apresentou ação protetora demonstrada pelos efeitos antioxidantes. Assim, o tratamento com a PDX pode ser um candidato promissor para aliviar parâmetros da condição diabética e melhorar comorbidades altamente incapacitantes, como dor neuropática e distúrbios emocionais associados ao DMT1.

Palavras-chave: estreptozotocina; mediadores lipídicos pró-resolução da inflamação; depressão; ansiedade; dor neuropática diabética; Protectina DX;

ABSTRACT

Protectin DX (PDX), a specialized pro-resolving lipid mediator, presents potential therapeutic applications across various medical conditions due to its anti-inflammatory and antioxidant properties. Since type-1 diabetes *mellitus* (T1DM) is a disease with inflammatory and oxidative profile, there is an urgent need to explore the use of PDX in addressing T1DM and its associated comorbidities, including diabetic neuropathic pain, depression, and anxiety. These conditions share common pathophysiological mechanisms, such as increased reactive oxygen species production. In the current study we investigated, in rats with induced T1DM, the PDX's effectiveness in alleviating neuropathic pain (mechanical allodynia; experiment 1), anxiety-like and depressive-like (experiment 2) behaviors. Also, we studied whether the PDX treatment would induce antioxidant effects in the hippocampus, prefrontal cortex, and blood plasma (experiment 3). After 2 weeks of T1DM induction with streptozotocin (50 mg/kg) in *Wistar* rats (110 individuals), PDX (1, 3, and 10 ng/animal; i.p. injection of 200 µl/animal) was administered continuously on days 14, 16, 18, 21, 24, and 27 after diabetes induction. All experiments were carried out in independent groups of rats. PDX consistently increased the mechanical threshold throughout the study at all doses, indicative of antinociceptive effect. For evaluating anxiety responses, animals were submitted to the elevated plus maze (day 26) and open field (day 28) tests, while for depressive-like behavior, the animals were tested in the modified forced swimming test (session test on day 28, with a pre-test session on day 27). All PDX doses effectively prevented the depressive-like and anxiety-like behaviors, compared to vehicle-treated T1DM rats. Beneficially, PDX significantly protected against oxidative stress in hippocampus, prefrontal cortex and blood plasma. Treated animals had ameliorated diabetic parameters by promoting weight gain and reducing hyperglycemia from T1DM rats. These findings suggest that PDX presents an antidepressant-like and anxiolytic-like action in animals with induced-T1DM, in addition to improving parameters related to the diabetic condition. It is worth noting that PDX also presented a protective action demonstrated by antioxidant effects. Thus, PDX treatment may be a promising candidate to alleviate parameters of the diabetic condition and improve highly disabling comorbidities such as neuropathic pain and emotional disturbances associated with T1DM.

Keywords: streptozotocin; specialized pro-resolving mediators; depression; anxiety; diabetic neuropathic pain; Protectin DX;

LISTA DE FIGURAS

LISTA DE FIGURAS DO ARTIGO

Fig. 6. Effect of the prolonged treatment with Protectin DX (PDX, 1, 3, or 10 ng/animal, i.p.) or vehicle (VEH, i.p.) in experimental-T1DM (STZ) or normoglycemic (VEH/VEH) rats on total reactive oxygen species (ROS, serum from blood samples). The injections took place on days 14, 15, 18, 21, 24, and 27 of the experimental protocol. Values were expressed as mean ± SEM (n = 5-7 rats/group). *p < 0.05 when compared to VEH/VEH; #p < 0.05 when compared to STZ/VEH…….57

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LISTA DE ABREVIATURAS OU SIGLAS

5-HT	Serotonina
AGPIs	Ácidos graxos poliinsaturados
ARA	Ácido araquidônico
COX	Ciclooxigenase
DHA	Ácido docosahexaenoido
DM	Diabetes *mellitus*
DMT1	Diabetes *mellitus* tipo 1
DMT2	Diabetes *mellitus* tipo 2
DND	Dor neuropática diabética
DP1	Receptores acoplados à proteína G DP
EPA	Ácido eicosapentaenoico
GPR	Receptor acoplado à proteína G
HGF	Fator de crescimento de hepatócitos
HPA	Eixo hipotálamo-pituitária-adrenal
IDF	International Diabetes Federation
IL	Interleucina
ISRS	Inibidores seletivos da recaptação de serotonina
LOX	Lipoxigenases
LX	Lipoxinas
LXR	Receptor X hepático
MaRs	Maresinas
MLPR	Mediadores lipídicos pró-resolução da inflamação
n-3	AGPIs da família ômega-3
n-6	AGPIs da família ômega-6
NA	Noradrenalina
NADPH	Nicotinamida adenina dinucleotídeo fosfato
NFκB	Fator nuclear kappa B
PD/NPD	Protectina/Neuroprotectina D
PD1	Protectina D1
PDX	Protectina DX
PMN	Leucócitos polimorfonucleares
PPAR	Receptores ativados por proliferadores de peroxissoma

ROS	Espécies reativas de oxigênio
RvDs	Resolvinas de série D
RvEs	Resolvinas de série E
STZ	Estreptozotocina
TGF- β	Fator de crescimento transformador-β
TNF-α	Fator de necrose tumoral-α

LISTA DE ABREVIATURAS OU SIGLAS DO ARTIGO

AMPK	Adenosine monophosphate-activated protein kinase
BDNF	Brain-derived neurotrophic factor
BG	Blood glucose
BSA	Bovine serum albumin
BW	Body weight
COX	Cyclooxygenases
DHA	Docosahexaenoic acid
DM	Diabetes *mellitus*
DNP	Diabetic neuropathic pain
EPA	Eicosapenaenoic acid
EPM	Elevated plus-maze test
HIP	Hippocampus
IL	Interleukin
LOX	Lipoxygenase
LPO	Lipid peroxidation
LPS	*E. coli* lipopolysaccharide
LRP	Lumbar radicular pain
LX	Lipoxin
MaRs	Maresin
mFST	Modified forced swim test
OFT	Open field test
PD/NPD	Protectin/Neuroprotectin D
PDX	Protectin DX
PFC	Prefrontal cortex

PUFAs	Polyunsaturated fatty acids
ROS	Reactive oxygen species
RvD5	Resolvin D5
RvDs	D-series resolvins
RvEs	E-series resolvins
SPMs	Specializes pro-resolving mediators
T1DM	Type-1 diabetes m*ellitus*
T2DM	Type-2 diabetes *mellitus*
TNF-α	Tumor necrosis factor-α
TST	Tail suspension test
VEH	Vehicle
WG	Weight gain

SUMÁRIO

1 INTRODUÇÃO

1.1 PROCESSO INFLAMATÓRIO E RESOLUÇÃO DA INFLAMAÇÃO

O processo inflamatório agudo ocorre nos seres vivos com a função de protegê-los de lesões teciduais e agentes infecciosos, além de promover a reparação de tecidos danificados por infecções ou traumas (Tabas e Glass, 2013). Este processo é composto de uma cascata complexa com diversos eventos fisiológicos organizados que levam à homeostase dos organismos, e é divida por especialistas em duas fases principais: estágio inicial e resolução (Serhan, 2014; Fullerton e Gilroy, 2016). O estágio inicial do processo inflamatório conta com processos vasculares e celulares, e é caracterizado por sinais clínicos: dor, rubor, calor, edema e perda de função (Freire e Dyke, 2000; Chen et al., 2017).

Os eventos vasculares consistem em, principalmente, vasodilatação e aumento da permeabilidade vascular, que resultam no extravasamento do exsudato fluido enriquecido em proteínas para o tecido danificado em que está o sítio inflamatório, acompanhado de infiltração celular, principalmente monócitos, basófilos, eosinófilos e neutrófilos (leucócitos polimorfonucleares – PMNs) (Abdulkhaleq et al., 2018). Além disso, são liberados uma série de mediadores e sinais inflamatórios que, por sua vez, dão continuidade ao processo inflamatório, tal como citocinas, quimiocinas, aminas vasoativas (histamina e serotonina) e eicosanoides (prostaglandinas, leucotrienos, tromboxanos) (Kotas e Medzhitov, 2015; Calder, 2020).

Após a eliminação dos estímulos inflamatórios, o próximo estágio é o processo de resolução da inflamação, que ocorre com o objetivo de retorno à homeostase do organismo (Serhan, 2014). Até o final do século 20, acreditava-se que a resolução da inflamação era um processo passivo. No entanto, a partir das descobertas do grupo liderado pelo Dr. Charles N. Serhan, que caracterizaram moléculas responsáveis por mediar essa etapa do processo inflamatório, começou-se a investigar a resolução da inflamação como um processo ativo (Freire e Dyke, 2000; Serhan, 2017a).

O processo inflamatório deve ser altamente controlado para que seja resolvido de maneira correta e haja retorno à homeostase. Quando há um desequilíbrio entre a resposta inflamatória e disfunções no processo resolutivo, a inflamação pode se prolongar e se tornar crônica, culminando em uma série de condições patológicas,

como doenças vasculares, distúrbios metabólicos e neurológicos (Kotas e Medzhitov, 2015; Serhan, 2017a). A Figura 1 tem como objetivo demonstrar a inflamação aguda de maneira resumida, com seus elementos e passos até a resolução ou cronificação deste processo (Serhan, 2017b).

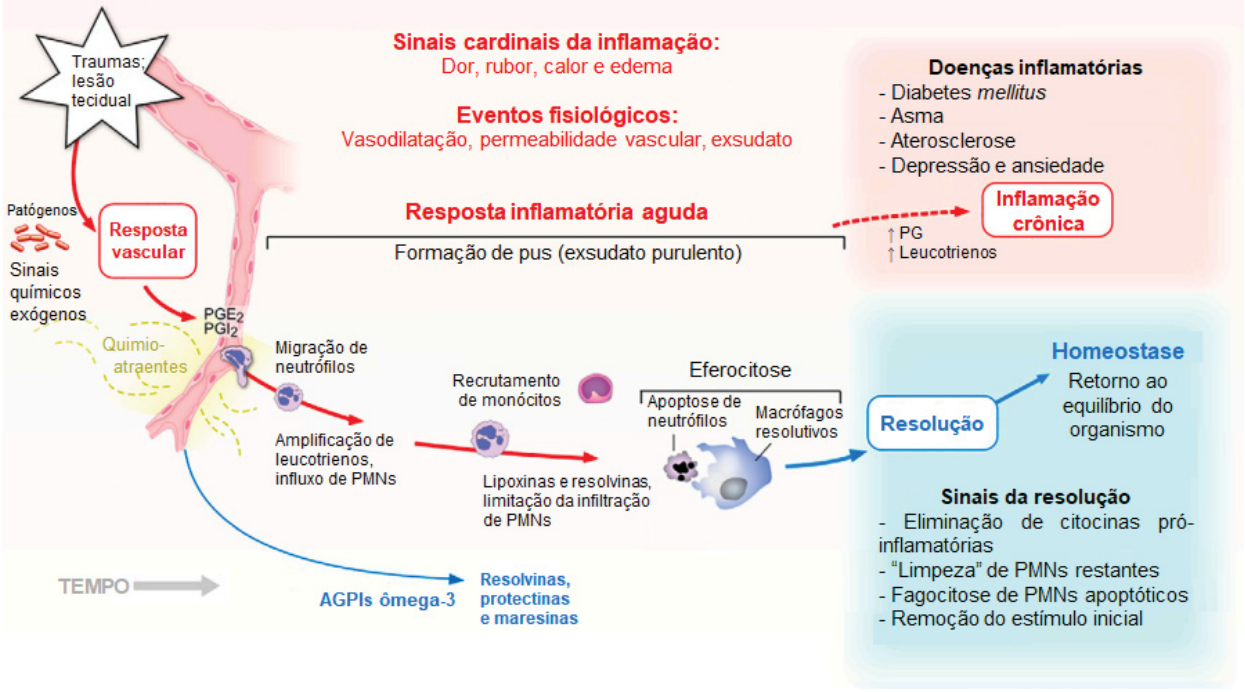

Figura 1. Elementos e passos do processo inflamatório agudo até resolução ou cronificação. Adaptado de Serhan, 2017b.

Para que a resolução seja eficaz, é essencial a eliminação dos agentes prejudiciais que deram início à inflamação aguda. Em infecções bacterianas, a remoção do patógeno é facilitada pela ação da nicotinamida adenina dinucleotídeo fosfato (NADPH) oxidase de PMNs, com a produção de espécies reativas de oxigênio (ROS) (Leto e Geiszt, 2006; Hahn et al., 2016). É importante ressaltar esta etapa em doenças autoimunes, como artrite reumatoide, lúpus eritematoso sistêmico e diabetes *mellitus* tipo 1 (DMT1), que são desencadeadas por antígenos endógenos (Abdolmaleki et al., 2019). A ativação de mediadores pró-inflamatórios e deficiências na resolução do processo inflamatório pode aumentar o risco e contribuir com o desenvolvimento de doenças autoimunes (Eizirik et al., 2009).

A resolução da inflamação também envolve uma série de eventos fisiológicos que incluem a interrupção da síntese de mediadores pró-inflamatórios e a degradação dos mediadores remanescentes da inflamação. Além disso, este estágio

inclui a redução da infiltração de neutrófilos e a eferocitose, que é o processo pelo qual os macrófagos degradam e eliminam as células apoptóticas resultantes do processo inflamatório (Fullerton e Gilroy, 2016; Cai et al., 2019; Yurdagul Jr et al., 2020). A eferocitose por si só também induz efeitos pró-resolutivos, aumentando níveis de mediadores pró-resolutivos, inibindo a óxido nítrico sintase induzível e promovendo a produção de fatores de crescimento angiogênicos (Doran et al., 2020). Estes efeitos ocorrem em parte pela ativação de receptores nucleares como o receptor X hepático (LXRα e LXRβ), responsável pelo metabolismo de carboidratos e lipídios e sinalização lipídica, além de ter função apoptótica via proteína quinase B - AKT) (Wang e Tontonoz, 2018). Outros receptores importantes na eferocitose são os receptores ativados por proliferadores de peroxissoma (PPARγ e PPARδ) que, quando ativado, realizam modulação imunológica mediada por células apoptóticas (Doran et al., 2020). Além disso, já foi demonstrado que a inibição da atividade de PPAR foi capaz de diminuir a expressão de fatores pró-resolutivos, como a interleucina (IL) anti-inflamatória IL-10, o fator de crescimento transformador-β (TGFβ) e o fator de crescimento de hepatócitos (HGF) (Yoon et al., 2015).

O processo de resolução da inflamação está representado na Figura 2, em que se observa os eventos tanto de maneira temporal quando a magnitude das repostas locais. A inflamação aguda dura de segundos a minutos, enquanto a resolução pode durar de horas a dias. Por fim, o momento pós-resolução antes do retorno à homeostase, inclui regeneração, cicatrização e reparação dos tecidos lesionados (Park et al., 2020).

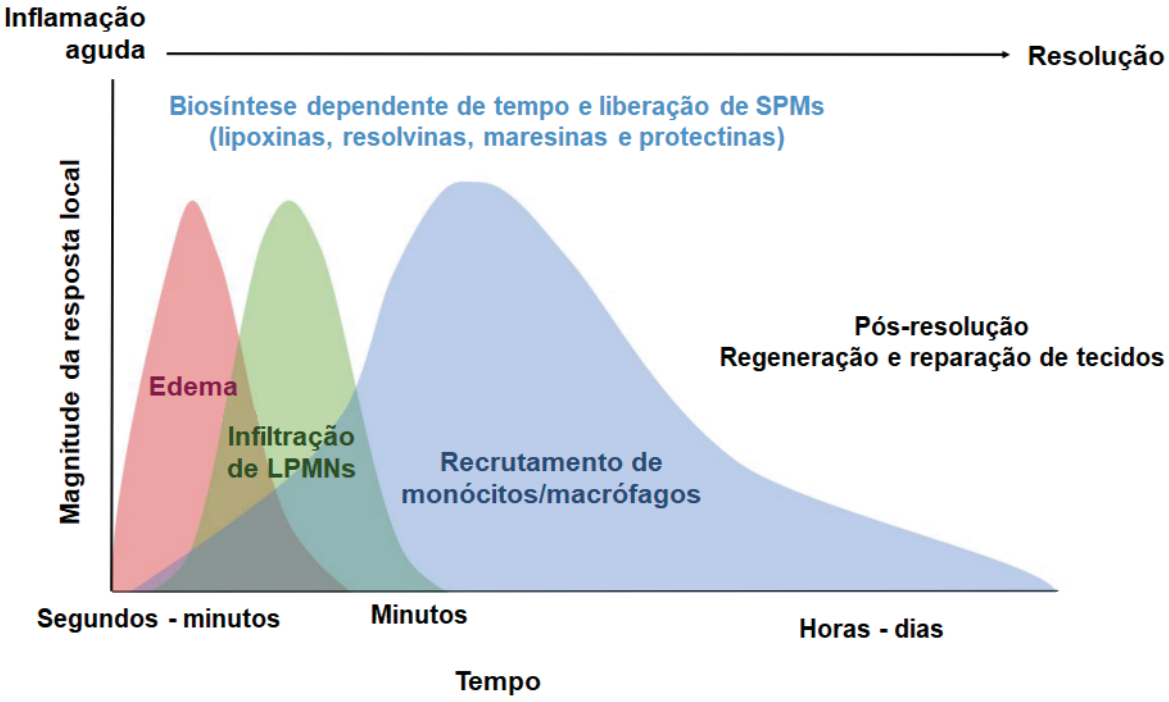

Figura 2. Inflamação aguda e resolução da inflamação. Adaptado de Park et al., 2020.

Além dos fatores que medeiam a resolução da inflamação já mencionados como a IL-10 e o TGFβ, existem outros fatores, como proteínas pró-resolutivas como a anexina A1, e principalmente os chamados mediadores lipídicos pró-resolução da inflamação: as lipoxinas, resolvinas, protectinas e maresinas (Tabas e Glass, 2013).

1.2 MEDIADORES LIPÍDICOS PRÓ-RESOLUÇÃO DA INFLAMAÇÃO E A PROTECTINA DX

Os mediadores lipídicos pró-resolução da inflamação (MLPR) são moléculas lipídicas bioativas essenciais no processo de resolução da inflamação para que haja retorno à homeostase após uma inflamação aguda, além de serem cruciais na reparação e regeneração de tecidos lesionados (Serhan, 2014, 2017a). Esses mediadores têm como precursores os ácidos graxos poli-insaturados (AGPIs) das famílias ômega-3 e ômega-6 (n-3 e n-6, respectivamente), e são sintetizados a partir das atividades enzimáticas de ciclooxigenases (COXs) e 5-lipoxigenase (5-LOXs), respectivamente (Dyall et al., 2022). Até o momento, várias séries de MLPR foram identificadas, e isso inclui as lipoxinas (LXs) derivadas do AGPI n-6 ácido araquidônico (ARA), resolvinas de série E (RvEs) do ácido eicosapentaenoico (EPA

– AGPI n-3), resolvinas de série D (RvDs), protectinas/neuroprotectinas (PD/NPD – de maior interesse neste estudo) e maresinas (MaRs), derivadas do ácido docosahexaenoico (DHA – AGPI n-3) (Serhan et al., 2011; Zhu et al., 2016).

Os MLPRs são produzidos a partir do metabolismo dos AGPIs n-3 (e n-6, no caso das lipoxinas) por enzimas específicas, incluindo as lipoxigenases (LOX) como a 5-LOX, 12-LOX e 15-LOX, COX, principalmente a enzima COX-2, e o citocromo P450 (CYP450) (Giacobbe et al., 2020). Este processo está demonstrado na Figura 3. As transformações enzimáticas que metabolizam os AGPIs em MLPRs ocorrem de maneira rápida dentro do organismo, e variantes genéticas (polimorfismos) das enzimas envolvidas neste processo (fosfolipase-A e COX) têm sido associadas a um risco aumentado de desenvolver transtornos psiquiátricos como a depressão induzida por interferon-α, que tem características inflamatórias pronunciadas (Papafili et al., 2002; Su et al., 2010; Hoyo-Becerra et al., 2014).

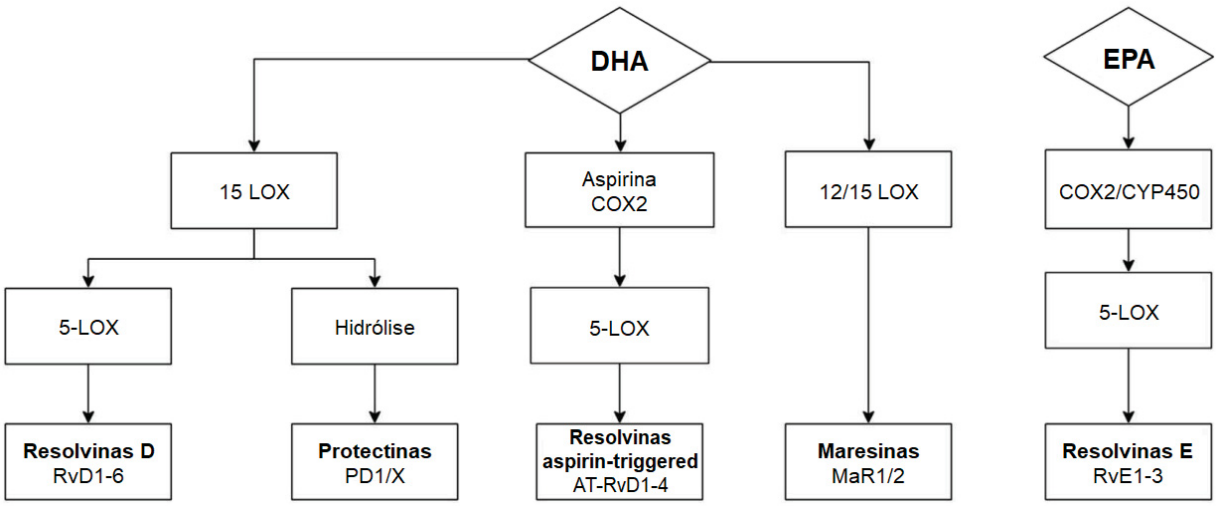

Figura 3. Metabolismo do EPA e do DHA em MLPRs a partir da atividade enzimática. Adaptado de Giacobbe et al., 2020.

A Protectina DX (PDX) ou ácido 10(S),17(S)-dihidroxi-E,Z,E-docosahexaenoico diHDoHE, de particular interesse neste estudo, pertence ao grupo de MLPR conhecido como protectinas. No mesmo grupo, encontramos também a Protectina D1 (PD1), sendo que PDX e PD1 são isômeros (Balas et al., 2017). A PDX é sintetizada a partir da dupla lipoxigenação do DHA, e apresenta atividade biológica reduzida em relação a mecanismos resolutivos quando comparada à PD1 (Guichardant et al., 2018). Mesmo assim, suas ações incluem a inibição da

agregação plaquetária em sangue de voluntários saudáveis, a redução da migração e extravasamento de neutrófilos, bem como a inibição da produção de ROS e da atividade da COX em neutrófilos humanos (Chen et al., 2011; Liu et al., 2014; Balas et al., 2017). Em outros estudos realizados *in vitro*, a PDX melhorou a sensibilidade à insulina e reduziu marcadores inflamatórios como o fator nuclear kappa B (NFκB) em adipócitos (Jung et al., 2017, 2018).

Em estudos com roedores, a PDX foi capaz de melhorar significativamente a sensibilidade à insulina e a inflamação em modelos de camundongos *db/db* diabéticos obesos e camundongos em dieta rica em gordura (White et al., 2014; Jung et al., 2017). Na literatura especializada, cada vez mais autores estão explorando a capacidade resolutiva e antidiabética da PDX, como apontado por estes e outros estudos *in vitro* e *in vivo*. Um estudo mais recente de 2023, demonstrou que a PDX preveniu o desenvolvimento de DMT1 e DMT2 induzidos experimentalmente em camundongos. A atividade antidiabética da PDX neste estudo pode ser explicada por sua ação antioxidante no plasma e pâncreas dos animais, anti-inflamatória no plasma e anti-apoptótica no tecido pancreático (Rengachar et al., 2023).

Em relação à atividade farmacológica da PDX, poucos estudos buscaram compreender a ação da PDX em receptores específicos até o presente momento. Enquanto estudos atribuem parte da ação da PD1 por atuar como agonista do receptor acoplado à proteína G 37 (GPR37) (Bang et al., 2018; Hansen e Serhan, 2022), apenas dois estudos apresentam atividade da PDX em receptores específicos. Um estudo de Hwang e colaboradores (2019) demonstrou em células endoteliais vasculares que expressam estresse oxidativo induzido por H_2O_2 que o estímulo com a PDX teve ação antioxidante. Um dos mecanismos propostos para a ação antioxidante foi a interação da PDX com o GPR 120, um receptor ativado por ácidos graxos de média e longa cadeia (Hara et al., 2009; Hwang et al., 2019). Esta interação foi testada a partir do uso de antagonistas do GPR 120, além de *knockdown* deste receptor nas células endoteliais. Nestes casos, o efeito protetor e antioxidante de PDX foi revertido (Hwang et al., 2019). Em outro estudo, publicado em 2022 por Hu e colaboradores, o tratamento com PDX promoveu a resolução da inflamação em um modelo de síndrome do desconforto respiratório agudo em ratos. Os autores sugerem que a resolução da inflamação pela PDX neste modelo ocorreu

pela ativação/interação com receptores acoplados à proteína G DP (DP1) (Hu et al., 2022; Hansen e Serhan, 2022).

Em resumo, a PDX tem sido explorada nos últimos anos por sua ação pró-resolutiva, protetora, antioxidante e antidiabética. Ainda assim, existem muitas lacunas na pesquisa não-clínica relacionada a este MLPR, visto que é um dos menos estudados em relação a outros como as Resolvinas, por exemplo. O entendimento mais abrangente dos MLPR e suas potenciais aplicações, incluindo a PDX, juntamente com o conceito emergente de resolução da inflamação, têm ampliado as possibilidades terapêuticas para tratar uma série de distúrbios com características inflamatórias, como o diabetes *mellitus,* de interesse para o presente estudo. O potencial terapêutico dos MLPR, como a PDX e outros, surge como uma área promissora de estudo não apenas no tratamento do diabetes *mellitus* em si e de comorbidades associadas, como dor neuropática e complicações cognitivas-emocionais, mas também o de outras doenças com características inflamatórias. Assim, a busca por tratamentos considerados protetores representa um avanço por se mostrarem mais eficazes e direcionados para essas doenças, podendo controlar o prognóstico melhorando ainda a qualidade de vida dos pacientes (Chiang e Serhan, 2017; Brennan et al., 2019; Leão et al., 2022; Dubé et al., 2022).

1.3 DIABETES *MELLITUS*

O diabetes *mellitus* (DM) é uma doença crônica e com caráter inflamatório e oxidativo, e tem como principal característica a hiperglicemia. O DM apresenta três principais tipos, o DM tipo-1 (DMT1), o DM tipo-2 (DMT2) e o DM gestacional, e essa classificação depende da maneira como a hiperglicemia se desenvolve em cada situação (*American Diabetes Association,* 2013). No DMT1, há a insuficiência na produção de insulina pelas células β-pancreáticas, ou seja, esta condição é insulinodependente e ocorre de maneira autoimune. Já no DMT2, a hiperglicemia ocorre por conta da resistência insulínica, ou ainda a falha na ação da insulina no organismo dos indivíduos. Por fim, o DM gestacional ocorre quando a hiperglicemia se desenvolve durante o período de gravidez, o que aumenta os riscos de desenvolvimento de DMT2 pela mãe e pelo bebê (Kerner e Brückel, 2014; Petersmann et al., 2022).

De acordo com a *International Diabetes Federation* (IDF, 2021), o número estimado de adultos (20 – 79 anos) com DM no ano de 2021 chegou a 537 milhões de pessoas no mundo, e há uma projeção de aumento de pelo menos 46% para os próximos 25 anos. É relevante destacar que uma parcela significativa dos adultos com diabetes desconhece seu diagnóstico, e em 2021, a porcentagem de indivíduos com diabetes não diagnosticada atingiu 44% globalmente (Ogurtsova et al., 2022). O DMT1, de particular interesse para este projeto, apresenta números preocupantes no número de casos existentes e reportados em pesquisas. O Brasil ocupa o 3º lugar no *ranking* de número de casos estimados de crianças e adolescentes com DMT1 em escala global, com aproximadamente 92,3 mil casos existentes e pelo menos 8,9 mil casos por ano nesta faixa etária (menores de 20 anos de idade) (IDF, 2021). Em adultos, por outro lado, os números relacionados ao DMT1 não são precisos devido a uma série motivos, incluindo diagnósticos incorretos como DMT2 ao invés de DMT1, além de, frequentemente, a coexistência de DMT2 e DMT1 no mesmo paciente (Cleland et al., 2013; Xu et al., 2018; Olamoyegun et al., 2020).

Em relação à etiologia do DMT1, esta condição é considerada uma doença autoimune, com três causas principais: predisposição genética, infecções virais e fatores ambientais (Acharjee et al., 2013; Norris et al., 2020). Devido à influência destes fatores, o sistema imunológico produz autoanticorpos que resultam na destruição das células β-pancreáticas, responsáveis pela produção de insulina. Este processo causa a deficiência insulínica, o que, por sua vez, leva ao desenvolvimento da hiperglicemia e do DMT1 (Atkinson et al., 2014; para uma revisão, ver Ilonen et al., 2019).

Para o estudo não-clínico do DMT1 em animais, um dos modelos mais amplamente adotados é induzido farmacologicamente pela estreptozotocina (STZ), um antibiótico sintetizado pela bactéria *Streptomyces achromogens* (King, 2012). A STZ possui uma estrutura molecular semelhante à glicose, o que a torna seletiva para os transportadores de glicose do tipo Glut-2, abundantemente encontrados nas células β-pancreáticas (Eleazu et al., 2013; Wu e Yan, 2015). Uma vez dentro das células, a STZ age como um agente alquilante de DNA, o que resulta em danos substanciais no material genético e contribui para a morte celular. Além disso, a STZ causa citotoxicidade ao elevar ROS e desencadear estresse oxidativo, que leva à morte celular, seguida pela eliminação das células β-pancreáticas pelos macrófagos (King, 2012; Wu e Yan, 2015). O impacto da STZ na população de células β-

pancreáticas é significativo, resultando em uma redução expressiva no número de células. Esse declínio na quantidade de células β-pancreáticas resulta em uma insuficiência de insulina e, como consequência, em hiperglicemia nos animais (Eleazu et al., 2013). Este processo de indução farmacológica do DMT1 pela STZ está representado na Figura 4 (Wu e Yan, 2015).

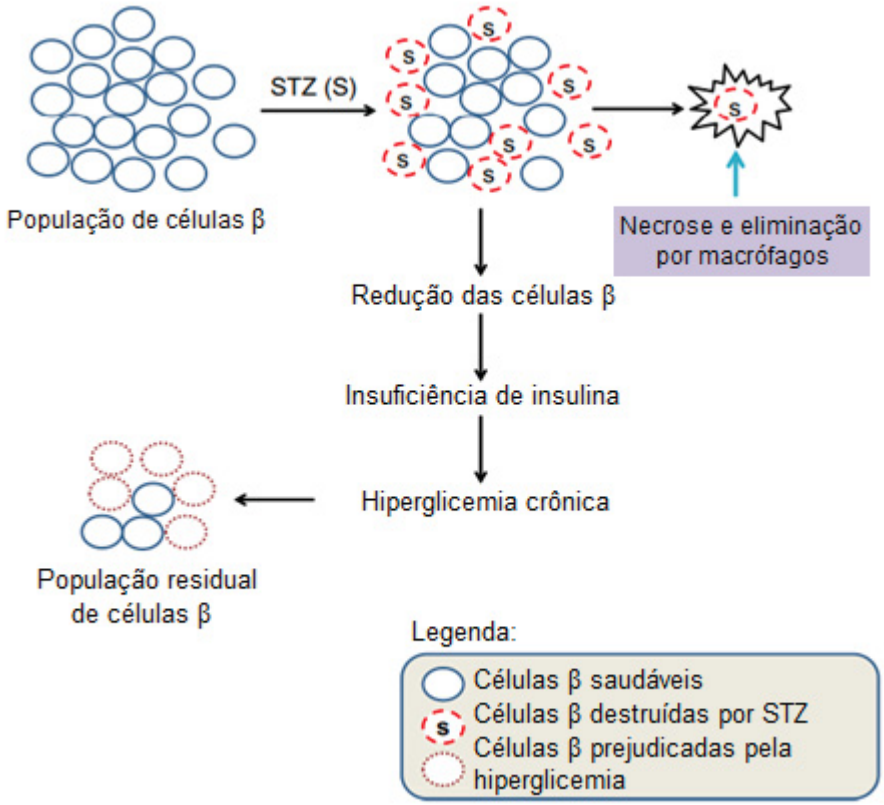

Figura 4. Ação da STZ sobre as células β-pancreáticas no modelo de indução experimental do DMT1. Adaptado de Wu e Yan, 2015.

A hiperglicemia do DMT1 ocasiona uma série de impactos adversos no organismo dos indivíduos, tanto em pacientes diabéticos quanto no modelo animal de DMT1 experimental. Por exemplo, os roedores com DMT1 experimental apresentam desregulação no eixo hipotálamo-pituitária-adrenal (HPA), indução expressiva de estresse oxidativo, prejuízos neuroquímicos (redução de monoaminas como serotonina (5-HT) e noradrenalina (NA) e quadros inflamatórios e oxidativos crônicos (com aumento de citocinas pró-inflamatórias, lipoperoxidação e alteração de antioxidantes endógenos, por exemplo) (de Morais et al., 2014; da Silva Dias et al., 2016; Pereira et al., 2018; Kang e Yang, 2020; Singh et al., 2020; Ribeiro et al., 2020; de Lima Silva et al., 2022; Chaves et al., 2023).

Em relação às consequências em âmbito clínico na vida dos indivíduos com DMT1, os sintomas e sinais estão presentes em pelo menos 90% dos pacientes, e consistem principalmente em perda de peso, poliúria (urina em excesso) e polidipsia (sede e consumo de água em excesso que levam à desidratação) (Dariya et al., 2019). O modelo animal de DMT1 induzido experimentalmente pela STZ é capaz de mimetizar estas características nos ratos/camundongos estudados (King, 2012). Além disso, em casos mais graves e avançados, principalmente sem diagnóstico, a cetoacidose diabética pode se desenvolver e, por conta disto, há a quebra de lipídios em cetonas como fonte alternativa de glicose, aumentando a acidose metabólica, o que pode levar inclusive à morte (Katsarou et al., 2017; Dariya et al., 2019).

Além dos sintomas e sinais relatados, os indivíduos com DMT1 podem desenvolver outras complicações, que são categorizadas em macro e microvasculares. Entre as complicações microvasculares específicas do DM, uma das principais é a neuropatia diabética, muito comum em sua forma dolorosa, a chamada dor neuropática diabética (Schreiber et al., 2015; Rosenberger et al., 2020). As complicações macrovasculares não são restritas ao DM, mas pacientes diabéticos correm maior risco de desenvolvê-las. Entre estas complicações estão predominantemente as doenças cardiovasculares (Katsarou et al., 2017; Dariya et al., 2019).

Outras comorbidades importantes do DM, são psiquiátricas, que tendem a ser mais prevalentes em pacientes com DM do que na população em geral e que são muitas vezes subdiagnosticadas (Roy e Lloyd, 2012; Zanoveli et al., 2016; Akbarizadeh et al., 2022). Entre as comorbidades psiquiátricas do DM (tanto tipo-1 quanto tipo-2), as mais comuns entre os pacientes e mais estudadas por pesquisadores são os transtornos de ansiedade e a depressão (Maia et al., 2012; Zanoveli et al., 2016; Buchberger et al., 2016). A ocorrência de comorbidades como a neuropatia diabética e transtornos psiquiátricos associados ao DM afeta a qualidade de vida dos pacientes de maneira extrema, com maior risco de prognósticos ruins e aumento de suicídio, o que ressalta a importância de estudos aprofundados relacionados a estas condições (Maia et al., 2012; Butwicka et al., 2015).

1.4 NEUROPATIA DIABÉTICA E DOR NEUROPÁTICA DIABÉTICA

A comorbidade mais comum associada ao DM é a neuropatia diabética, uma condição que afeta nervos periféricos sensoriais e autonômicos (Feldman et al., 2017; Rosenberger et al., 2020). A principal consequência da neuropatia diabética é a condição em sua forma dolorosa conhecida como dor neuropática diabética (DND) (Schreiber et al., 2015; Redivo et al., 2016; Jesus et al., 2019; Rosenberger et al., 2020). A dor associada à DND é frequentemente caracterizada pelos pacientes por sensações de queimação, choques elétricos, formigamento e pode também incluir alodinia, que é a dor devido a um estímulo que em condições normais não causa sensação dolorosa, e hiperalgesia, sensação dolorosa intensa de maneira anormal após estímulo doloroso. A convivência diária com a DND diminui significativamente a qualidade de vida dos indivíduos afetados (Schreiber et al., 2015; Zeng et al., 2017; Zhang et al., 2022).

O DM pode causar uma série de danos no sistema nervoso periférico, sendo que o dano mais comum ocorre de maneira simétrica e bilateral, acometendo principalmente nervos dos pés e mãos (Feldman et al., 2017). A gravidade dos danos segue um padrão comum nos pacientes chamado de “neuropatia em bota e luva”, em que há alterações de sensibilidade e dor principalmente nestas regiões (Jensen et al., 2021).

A fisiopatologia da DND ainda não é completamente compreendida, mesmo que várias teorias tenham sido propostas para explicar a dor relacionada à neuropatia diabética. Algumas destas teorias propostas incluem alterações em vasos sanguíneos que irrigam nervos periféricos, alterações metabólicas e autoimunes, como a hiperglicemia característica do DM, ativação de células da glia devido a condições inflamatórias e mudanças na expressão de canais de sódio e canais de cálcio (Tesfaye et al., 2013; Schreiber et al., 2015). De qualquer maneira, é um consenso que a hiperglicemia é um fator crucial para o desenvolvimento da DND (Oyibo et al., 2002; Alay et al., 2022).

Além do papel da hiperglicemia no desenvolvimento da DND, outra via metabólica importante é a via dos polióis. Quando há a hiperativação desta via devido ao excesso de glicose sanguínea, há a conversão de glicose em sorbitol e, posteriormente, frutose – produtos metabólicos da via dos polióis (Schreiber et al., 2015). Estas conversões ocorrem a partir da atividade enzimática da aldose

redutase e da sorbitol desidrogenase, respectivamente, e conta com o consumo de NADPH e NAD+ (Dewanjee et al., 2018). A redução exacerbada de NADPH pode levar a uma indução de estresse oxidativo, visto que o NADPH é um componente importante na produção do tripeptídeo antioxidante glutationa reduzida. Além disso, o acúmulo de sorbitol e frutose leva à redução da concentração de vitaminas do complexo B, inibição da bomba de Na^+/K^+-ATPase e acúmulo de sódio intracelular (Zenker et al., 2013). Além do estresse oxidativo causado a partir da via dos polióis com a diminuição nos níveis de enzimas antioxidantes, o que causa desequilíbrio entre produção de ROS e agentes antioxidantes, outros mecanismos são explorados na literatura (Singh et al., 2020). O estresse oxidativo relacionado à DND também pode ser desencadeado a partir de outras vias, tais como a oxidação da glicose em excesso e seus metabólitos, alterações na função mitocondrial e hiperativação das isoformas da proteína quinase C, ativado pelo diacilglicerol que está aumentado devido à hiperglicemia (Schreiber et al., 2015; Kang e Yang, 2020).

Por conta da fisiopatologia complexa, multifatorial e não completamente esclarecida da DND, o gerenciamento eficaz desta condição, tanto por meio de abordagens farmacológicas quanto não farmacológicas, é um desafio significativo para os pacientes (Jang e Oh, 2023). O tratamento da DND é realizado de maneira a aliviar os sintomas, e apenas três agentes terapêuticos são aprovados pelo FDA para o tratamento: o anticonvulsivante pregabalina, o inibidor da recaptação de serotonina e noradrenalina, duloxetina, e o agonista de receptores opioides e inibidor da recaptação de noradrenalina tapentadol (Schreiber et al., 2015). Com a resolução da inflamação sendo cada vez mais explorada em estudos relacionados a dor e ao DM, e devido aos mecanismos fisiopatológicos propostos relacionados ao estresse oxidativo, a utilização de MLPR na DND parece ser muito promissora.

1.5 ANSIEDADE E DEPRESSÃO ASSOCIADAS AO DIABETES *MELLITUS*

Outra complicação importante associada ao DM é a encefalopatia diabética, termo utilizado por alguns neurocientistas para se referir aos característicos comprometimentos cognitivos-emocionais, bem como às alterações estruturais, moleculares e neuroquímicas de áreas encefálicas importantes para a modulação de comportamentos que envolvam as emoções, julgamento e processamentos de memória, como o hipocampo e córtex pré-frontal. Essa encefalopatia diabética

facilita ou precipita o surgimento de doenças psiquiátricas (Wayhs et al., 2014; de Morais et al., 2014; Zhai et al., 2018; Chen et al., 2018; Ribeiro et al., 2020; de Lima et al., 2022; Leão et al., 2022; Waltrick et al., 2023). De principal interesse para este estudo, tem sido discutido e demonstrado uma relação bidirecional entre transtornos de humor como depressão e transtornos de ansiedade com o DM, na qual diversos mecanismos neurobiológicos se interligam (Wayhs et al., 2014; Zanoveli et al., 2016). É também importante ressaltar que há a presença de um fator psicológico ligado ao DM e a toda a demanda que esta condição exige do indivíduo (Boden, 2018).

Entre os mecanismos compartilhados pelo DM e pela depressão e ansiedade, estão o estresse oxidativo e a inflamação, com base em uma série de evidências que demonstram a superprodução de espécies reativas de oxigênio/nitrogênio e níveis elevados de marcadores pró-inflamatórios como interleucina (IL)-1β e fator de necrose tumoral-α (TNF-α) (Giacco e Brownlee, 2010; de Morais et al., 2014; da Silva Dias et al., 2016; Wang et al., 2016; Zhai et al., 2018; Pereira et al., 2018; dos Santos et al., 2021; Leão et al., 2022). A depressão e a ansiedade, bem como o DM, induzem alterações comuns importantes no sistema imunológico, como neuroinflamação, aumento de citocinas pró-inflamatórias e inflamações crônicas. Um estudo feito em 2012 por Hood e colaboradores mostrou que pacientes com depressão associada ao DM apresentaram um aumento significativo de agentes pró-inflamatórios (Hood et al., 2012), como a lipoproteína A, uma lipoproteína capaz de ativar macrófagos e monócitos e aumentar a produção de citocinas pró-inflamatórias (Orsó e Schmitz, 2017).

Em relação à ansiedade, a neuroinflamação também é um componente importante na fisiopatologia deste transtorno. Vários estudos, clínicos e não-clínicos mostraram o aumento de citocinas pró-inflamatórias em pacientes ansiosos e em animais com comportamento tipo-ansioso, demonstrando uma relação entre a neuroinflamação com a ansiedade (Salim et al., 2012; Simões et al., 2018). A literatura também traz o estresse oxidativo como gatilho para a inflamação crônica que pode desencadear psicopatologias como a ansiedade, com o aumento de ROS, citotoxicidade e morte celular (Wang et al., 2021). O estresse oxidativo aumenta a neuroinflamação a partir do aumento de citocinas pró-inflamatórias tais como algumas interleucinas, TNF-α, como também pelo aumento de fatores de transcrição como o NFκB (Salim et al., 2012; Hou e Baldwin, 2011).

Tendo em vista todas a complicações da depressão e ansiedade associadas ao DM, um grande desafio para os pacientes diabéticos é o tratamento dessas psicopatologias. Geralmente, os medicamentos de primeira escolha para o tratamento da depressão e ansiedade associadas ao DM são os antidepressivos monoaminérgicos, como a fluoxetina e a sertralina (inibidores seletivos da recaptação de serotonina - ISRS) (Huang et al., 2013; Zanoveli et al., 2016). Muitos destes pacientes diabéticos são resistentes e/ou refratários a estes medicamentos, ou seja, não respondem bem ao tratamento. Os antidepressivos normalmente utilizados também podem precipitar efeitos colaterais indesejáveis, como alterações na pressão arterial, alterações de peso e disfunção sexual, e estes efeitos adversos podem inclusive acarretar a descontinuidade do uso do medicamento (Roopan e Larsen, 2016). O uso de antidepressivos por pacientes diabéticos causa ainda outro problema: alguns antidepressivos utilizados podem prejudicar o controle glicêmico, além de interagir com drogas hipoglicemiantes (Barnard et al., 2013; Gagnon et al., 2018). Em um estudo de 2018, Gagnon e colaboradores mostraram os efeitos dos antidepressivos utilizados na clínica no controle glicêmico dos pacientes diabéticos, inclusive alertando para o fato de que mesmo antidepressivos diferentes dentro da mesma classe, como os ISRS, podem alterar o controle glicêmico de formas distintas (Gagnon et al., 2018).

Dada a natureza inflamatória e oxidativa do DM (Wang et al., 2016) e as comorbidades associadas, incluindo DND e comprometimentos emocionais-cognitivos, além o desafio no tratamento destes transtornos psiquiátricos, existe uma necessidade urgente de estudos que explorem o potencial terapêutico dos MLPR no tratamento destas condições.

2 OBJETIVOS

2.1 OBJETIVO GERAL

O objetivo geral deste estudo foi avaliar em ratos com DMT1 induzido experimentalmente o efeito do tratamento prolongado com a Protectina DX (PDX) sobre comportamentos relacionados à dor neuropática, à depressão e à ansiedade, bem como sobre a condição diabética em si e sobre parâmetros de estresse oxidativo no córtex pré-frontal, hipocampo e plasma sanguíneo dos animais.

2.2 OBJETIVOS ESPECÍFICOS

Os objetivos específicos deste estudo foram:

- Avaliar o efeito do tratamento prolongado com a PDX em três doses (1, 3 e 10 ng/animal; i.p. com volume de 200 µl/animal) sobre a condição diabética em ratos com DMT1 experimental por meio da análise da glicemia sanguínea e ganho de peso dos animais;
- Avaliar o efeito do tratamento prolongado com a PDX em três doses (1, 3 e 10 ng/animal; i.p. com volume de 200 µl/animal) sobre a alodinia mecânica em ratos com DMT1 experimental submetidos ao teste do Von Frey eletrônico;
- Avaliar o efeito do tratamento prolongado com a PDX em três doses (1, 3 e 10 ng/animal; i.p. com volume de 200 µl/animal) sobre o comportamento tipo-ansioso em ratos com DMT1 experimental submetidos aos testes do campo aberto e labirinto em cruz elevado;
- Avaliar o efeito do tratamento prolongado com a PDX em três doses (1, 3 e 10 ng/animal; i.p. com volume de 200 µl/animal) sobre o comportamento tipo-depressivo em ratos com DMT1 experimental submetidos ao teste de natação forçada modificado;
- Avaliar o efeito do tratamento prolongado com a PDX em três doses (1, 3 e 10 ng/animal; i.p. com volume de 200 µl/animal) sobre parâmetros de estresse oxidativo por meio da análise de níveis de ROS no córtex pré-frontal, hipocampo e plasma sanguíneo (soro), e lipoperoxidação (LPO) no córtex pré-frontal e hipocampo de ratos com DMT1 induzido experimentalmente;

3 ARTIGO CIENTÍFICO

TITLE PAGE

Protectin DX treatment induces beneficial and protective effects on parameters related to behavior and the type-1 diabetes *mellitus* conditions: a non-clinical approach

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Highlights

- Protectin DX induced antinociceptive effect T1DM rats;
- Protectin DX improved weight gain and glycemia index of T1DM rats;
- Protectin DX prevented anxiety-like behavior in rats with T1DM;
- Protectin DX prevented depressive-like behavior in rats with T1DM;
- Protectin DX reduced oxidative stress parameters in rats with T1DM.

Abstract

Protectin DX (PDX), a specialized pro-resolving lipid mediator, presents potential therapeutic applications across various medical conditions due to its anti-inflammatory and antioxidant properties. Since type-1 diabetes *mellitus* (T1DM) is a disease with inflammatory and oxidative profile, there is an urgent need to explore the use of PDX in addressing T1DM and its associated comorbidities, including diabetic neuropathic pain, depression, and anxiety. These conditions share common pathophysiological mechanisms, such as increased reactive oxygen species production. In the current study we investigated, in rats with induced T1DM, the PDX's effectiveness in alleviating neuropathic pain (mechanical allodynia; experiment 1), anxiety-like and depressive-like (experiment 2) behaviors. Also, we studied whether the PDX treatment would induce antioxidant effects in the hippocampus, prefrontal cortex, and blood plasma (experiment 3). After 2 weeks of T1DM induction with streptozotocin (50 mg/kg) in *Wistar* rats, PDX (1, 3, and 10 ng/animal; i.p. injection of 200 µl/animal) was administered continuously on days 14, 15, 18, 21, 24, and 27 after diabetes induction. All experiments were carried out in independent groups of rats. PDX consistently increased the mechanical threshold throughout the study at all doses, indicative of antinociceptive effect. For evaluating anxiety responses, animals were submitted to the elevated plus maze (day 26) and open field (day 28) tests, while for depressive-like behavior, the animals were tested in the modified forced swimming test (day 28, with a pre-test session on day 27). All PDX doses effectively prevented the depressive-like and anxiety-like behaviors, compared to vehicle-treated T1DM rats. Beneficially, PDX significantly protected against the oxidative stress parameters in hippocampus, prefrontal cortex and blood plasma. Treated animals had ameliorated diabetic parameters by promoting weight gain and reducing hyperglycemia from T1DM rats. These findings suggest that PDX presents an antidepressant-like and anxiolytic-like action in animals with induced-T1DM, in addition to improving parameters related to the diabetic condition. It is worth noting that PDX also presented a protective action demonstrated by antioxidant effects. Thus, PDX treatment may be a promising candidate to alleviate parameters of the diabetic condition and improve highly disabling comorbidities such as neuropathic pain and emotional disturbances associated with T1DM.

Keywords: streptozotocin; specialized pro-resolving mediators; depression; anxiety; diabetic neuropathic pain; Protectin DX.

1. Introduction

Specialized pro-resolving mediators (SPMs) constitute a category of bioactive lipid molecules crucial for orchestrating the process of inflammation resolution and facilitating tissue repair. These mediators originate from n-3 and n-6 polyunsaturated fatty acids (PUFAs) through the enzymatic activities of cyclooxygenases (COXs) and 5-lipoxygenase (5-LOXs), respectively (Dyall et al., 2022). They are synthesized during the later phases of an inflammatory response and play a pivotal role in regulating the restoration of tissue homeostasis following immune activation (Zaninelli et al., 2021; Abdolmaleki et al., 2020). Currently, various series of SPMs have been identified, including lipoxins (LXs) derived from arachidonic acid, E-series resolvins (RvEs) from eicosapentaenoic acid (EPA), D-series resolvins (RvDs), protectin/neuroprotectin (PD/NPD), and maresins (MaRs) from docosahexaenoic acid (DHA) (Serhan et al., 2011; Zhu et al., 2016). The discovery and comprehensive understanding of SPMs, coupled with the concept of inflammation resolution, have opened novel therapeutic avenues for addressing a wide spectrum of inflammatory and immune-related disorders, including diabetes *mellitus* (DM) (Chiang and Serhan, 2017; Brennan et al., 2019; Leão et al., 2022; Dubé et al., 2022).

Of interest to the present study, DM is a disease of significant global prevalence in which data from the International Diabetes Federation (10th edition of the Diabetes Atlas) highlighted that 537 million adults are currently living with DM, and this number does not include individuals with DM but that remains underdiagnosed (IDF, 2021). With hyperglycemia as its primary characteristic leading to complications like oxidative stress and the onset of chronic inflammation, DM presents a series of harm and dysfunctions in various tissues, including both central and peripheral nervous systems (Zanoveli et al., 2016; Rosenberger et al., 2020; IDF, 2021). As a result of these complications, DM leads to a range of severe comorbidities, including diabetic neuropathy and diabetic encephalopathy (Redivo et al., 2016; Zanoveli et al., 2016; Jesus et al., 2019; IDF, 2021; Leão et al., 2022; Chaves et al., 2023).

The primary outcome of diabetic neuropathy is the painful condition known as diabetic neuropathic pain (DNP) (Schreiber et al., 2015; Jesus et al., 2019; Rosenberger et al., 2020) that is often characterized as burning, electric shock-like sensations, tingling, and may also include allodynia, along with significantly

decreased quality of life (Schreiber et al., 2015; Zeng et al., 2017; Zhang et al., 2022). Effectively managing DNP, both through pharmacological and non-pharmacological means, is a significant challenge for patients, primarily because of its complex and multifactorial pathophysiology (Jang and Oh, 2023).

Another important complication associated with DM is diabetic encephalopathy characterized by cognitive impairments, structural brain changes, leading to psychiatric disorders (Wayhs et al., 2014; Zanoveli et al., 2016; Zhai et al., 2018; Chen et al., 2018, Chaves et al., 2023). Of main interest to this study, depression and anxiety related disorders are linked to DM through a bidirectional relationship in which numerous neurobiological mechanisms overlap (Wayhs et al., 2014; Zanoveli et al., 2016). Among the mechanisms shared by these conditions are oxidative stress and inflammation, supported by a series of evidence demonstrating an increase of the reactive oxygen/nitrogen species production and/or reduced antioxidant levels associated with elevated levels of pro-inflammatory markers like interleukin (IL)-1β and tumor necrosis factor-α (TNF-α) (Koo and Duman, 2008, 2009; Giacco and Brownlee, 2010; de Morais et al., 2014; da Silva Dias et al., 2016; Wang et al., 2016; Liu et al., 2018; Zhai et al., 2018; dos Santos et al., 2021; Leão et al., 2022).

Given the inflammatory and oxidative components of DM (de Morais et al., 2014; Wang et al., 2016; Pereira et al., 2018; Leão et al., 2022; de Lima Silva et al., 2022) and the associated consequences, including emotional and cognitive impairments as well as DNP (Wayhs et al., 2014; Redivo et al., 2016, 2019; Jesus et al., 2019; Gasparin et al., 2021; Waltrick et al., 2023; Jang and Oh, 2023; Lu et al., 2023), there is an urgent need for studies exploring the therapeutic potential of SPMs. In this regard, our laboratory previously demonstrated in a rat model of type-1 DM (T1DM) that long-term supplementation with fish oil, rich in n-3 PUFAs (EPA and DHA), effectively prevented the development of mechanical allodynia and depressive-like behaviors related to T1DM. Additionally, this supplementation preserved the levels of brain-derived neurotrophic factor (BDNF) in the hippocampus (HIP) and prefrontal cortex (PFC), both of which are brain regions implicated in regulating emotional and cognitive responses (Redivo et al., 2016). Interestingly, Redivo and colleagues (2019) demonstrated that acute oral supplementation with either EPA or DHA during the 2nd or 4th week following the induction of T1DM in rats

significantly alleviated mechanical allodynia in these animals. Moreover, subchronic supplementation elicited a sustained antinociceptive effect (Redivo et al., 2019).

More recently, our laboratory conducted a study to assess the therapeutic effect of the SPM Resolvin D5 (RvD5) on depressive-like and anxiety-like behaviors in rats with induced-T1DM (Leão et al., 2022). After prolonged treatment with RvD5 in these animals with induced T1DM, the therapy mitigated the more pronounced anxiety-like responses observed in rats with experimental T1DM, while improving depressive-like behaviors, which are also more pronounced in these animals. RvD5 treatment also significantly improved blood glucose levels and weight gain in these T1DM rats, indicating a prevention or reduction in the severity of parameters related to the diabetic condition Furthermore, the treatment prevented the elevation of pro-inflammatory cytokine IL-1β levels in the HIP and PFC of rats with experimental T1DM, reinforcing the protective profile of the SPMs (Leão et al., 2022).

Regarding PDX, of interest to this study, studies demonstrate its anti-inflammatory role, inhibiting COX-1 and COX-2 enzymes, while also significantly reducing the production of reactive oxygen species (ROS) and the release of myeloperoxidase, indicating protective properties (Liu et al., 2014; Lagarde et al., 2020; Wang et al., 2020). Zhao and colleagues conducted a study in 2022 in which they assessed the analgesic effects of PDX and its potential mechanisms in rats with lumbar radicular pain due to non-compressive lumbar disc herniation, a frequent form of chronic pain disorder (Zhao et al., 2022). Administration of PDX (10 ng or 100 ng; 10 µl via intrathecal) resulted in a reduction in the mRNA levels of pro-inflammatory cytokines IL-6 and IL-1β, as well as facilitated the transcription of mRNA for transforming growth factor β1, an anti-inflammatory factor that can produce an analgesic effect in painful conditions (Chen et al., 2013). This pharmacological intervention with PDX also resulted in a reduction in mechanical and thermal hyperalgesia in the rat model of lumbar radicular pain. When the nucleus pulposus was implanted to the rats' dorsal root ganglion to induce the herniated disc inherent to the model, there was an interruption in autophagy flow and adenosine monophosphate-activated protein kinase (AMPK) signaling in the spinal dorsal horns. PDX treatment demonstrated a dose-dependent ability to restore impaired autophagy flux and AMPK signaling (Zhao et al., 2022). More recently and of interest for the study, Rengachar and colleagues (2023) demonstrated that PDX prevented the development of experimentally induced T1DM and DMT2 in male Swiss mice.

Furthermore, treatment with PDX (50 ng/animal; intraperitoneal route) exhibited highly significant anti-inflammatory, antioxidant, and antiapoptotic effects, which could account for the antidiabetic properties of PDX (Rengachar et al., 2023).

As rats with experimentally-induced T1DM exhibit oxidative stress in the lumbar spinal cord, sciatic nerve, besides limbic and cortical areas crucial for mediating emotional responses (for a review, see Muriach et al., 2014; de Morais et al., 2014; Pereira et al., 2018; Gasparin et al., 2021; de Lima Silva et al., 2022), the evaluation of a likely antioxidant profile of PDX becomes relevant for studies using this animal model of DM. Taking into account the exposed above, it is crucial to initiate research aiming to explore the therapeutical potential of PDX and similar compounds as promising agents to treat DM comorbidities besides DM parameters itself.

Therefore, the primary objective of this study was to investigate the effects of PDX on DNP and the frequently associated emotional alterations to T1DM, such as anxiety-like and depressive-like behaviors, in rats with experimentally induced T1DM. Additionally, this study assessed a likely protective effect of PDX by evaluating parameters related to the oxidative stress in the HIP, PFC and blood plasma (serum) from T1DM animals.

2. Material and Methods

2.1. Ethics statements

This study was performed at the Federal University of Paraná and followed all National Council for the Control of Animal Experimentation (CONCEA) recommendations. All experiments carried out were approved by the local Ethics Committee on Animal Experimentation (license number: CEUA/BIO - UFPR; #1108) and the necessary efforts were made to minimize animal suffering, as well as to reduce the number of animals used.

2.2. Animals

Male *Wistar* rats (aged 8-10 weeks, n = 110 animals) were used in the experiments, provided by the *vivarium* of the Federal University of Parana. The animals were kept 4 per cage (41 × 32 × 16.5 cm) in a temperature-controlled room (22 ± 2 ◦ C), on a 12-hour light-dark cycle and with food and water *ad libitum*. All the

experimental procedures were performed during the light phase of the cycle (7:00 am to 7:00 pm). Behavioral experiments were performed by two of the experimenters (APFW, FAAB), while analysis of oxidative stress parameters was performed by more two other experimenters (DRR, KMO, APFW). Video analysis was performed by blinded experimenters (APFW, FAAB).

2.3. Chemical reagents

In this study, the following drugs were used: streptozotocin (STZ, Cayman Chemicals, USA) and Protectin DX (PDX, Cayman Chemicals, USA). STZ (60 mg/kg) was dissolved in a citrate buffer and injected intraperitoneally (i.p.) for T1DM induction. PDX was diluted in sterile saline (0.9% NaCl) at doses of 1, 3 and 10 ng/animal (i.p. injection of 200 µl/animal). The doses chosen for this study were based on doses found in the literature in studies with other SPMs, since this is the first study to evaluate the treatment with PDX in the behaviors evaluated in *Wistar* rats (Fattori et al., 2019; Leão et al., 2022; Baggio et al., 2023). The rats that were part of the control groups in the study received sterile saline as a vehicle.

2.4. Experimental type-1 diabetes mellitus induction (T1DM)

To carry out the experimental T1DM induction, the animals underwent a 12-hour fast before the procedure. After fasting, a single injection of STZ (60 mg/kg, i.p.) was performed in the animals that were part of the experimental groups (STZ). In the control group of normoglycemic animals, a single injection of the vehicle (citrate buffer, 10 mM, pH 4.5) was administered. After 72 hours of this procedure, the diabetic condition was confirmed by adding blood samples (5 µL) from the tail vein of the animals to test strips impregnated with glucose oxidase (Accu-Check Active™, Roche). Rats were considered hyperglycemic when blood glucose was equal to or greater than 250 mg/dL and maintained in the study in experimental STZ groups (Leão et al., 2022; Waltrick et al., 2023).

2.5. Behavioral tests

Behavioral tests were performed to assess anxious-like and depressive-like behaviors in addition to mechanical allodynia. On the day of each test, the animals were allocated in the experimentation room for at least 30 minutes for acclimatization. The tests were recorded with a Sony action cam 4K camera for later

analysis and performed by experimenters blinded to the treatment conditions (APFW, FAAB).

2.5.1. Mechanical allodynia measurements (electronic Von Frey test)

Mechanical allodynia was assessed using an electronic Von Frey (Insight Equipamentos, Ribeirão Preto, SP, Brazil). The device consisted of a disposable polypropylene tip (0.5 mm in diameter) connected to a force transducer, which was in turn connected to a digital display for measuring the force (in grams) applied to the medial surface of the hind legs of the rats. Plexiglass boxes were placed on a wire mesh floor (5 mm² thick) were used to acclimate the rats for 15 minutes until they ceased exploratory behaviors. To conduct the test, an increasing force was applied linearly across the gaps in the wire mesh using the disposable tip, aiming to induce a withdrawal response or "shake/lick" in the stimulated paw. The stimulus was performed until three similar withdrawal responses were achieved to calculate the mechanical threshold of a single paw. The average of these three values was then calculated.

For calculating the mechanical threshold of an animal, the mean value of the previously calculated mechanical thresholds of the two hind paws of each rat was used. To determine the presence of mechanical allodynia, the difference between the mean mechanical thresholds observed before STZ administration (baseline) and different time points after T1DM induction or after treatment with PDX was calculated (Redivo et al., 2016; Jesus et al., 2019; Gasparin et al., 2021).

2.5.2. Elevated plus maze (EPM) test

The EPM was used to assess the potential anxiolytic-like effect of PDX treatment. The apparatus consisted of a wooden structure 50 cm above the ground, with four arms (two open and two closed with walls) and a central area of 10 cm². To carry out the experiment, each animal was placed in the center of the apparatus with its snout facing a closed arm and was allowed to explore freely for 5 minutes.

As an anxiety index, the number of entries and the percentage of time in the open arms of the apparatus were quantified. The number of entries into the closed arms were quantified as an assessment of the rats' locomotor activity. Additionally, ethological measures were investigated, including the frequency of head dipping (an exploratory movement in the open arms where the animal projects its head towards

the floor) and risk assessment (exiting an enclosed arm with the forepaws and head and investigating the surroundings). Between each test session, the apparatus was cleaned with a 20% ethanol solution (Cruz et al., 1994; Silva and Brandão, 2000; Chaves et al., 2021; de Lima Silva et al., 2022; Waltrick et al., 2023).

2.5.3. Open field test (OFT)

The open field apparatus consisted of a rectangular box (40 cm × 50 cm × 63 cm) divided into 9 square units. This test was used to assess locomotor and exploratory activity as well as some parameters related to anxiety. The rats were individually placed in the center of the apparatus for 5 minutes, and their behaviors were recorded for subsequent analysis. To assess locomotor activity, the number of crossings made with all four paws from one unit to another was quantified. As for exploratory activity, the frequency of grooming (self-cleaning) and rearing (rising on the hind limbs) were also quantified. As anxiety parameter, the time spent in the center of the apparatus (in seconds) was measured (Waltrick et al., 2022, Leão et al., 2022).

2.5.4. Modified forced swim test (mFST)

To evaluate the antidepressant-like effect of PDX treatment, the mFST was performed. The apparatus consisted of an acrylic cylinder (20 cm x 50 cm) containing a 30 cm column of water (24±1 °C). In this protocol, a pre-test session was performed, in which the rats were placed individually in the cylinder for 15 minutes. 24 hours after the pre-test, the rats were submitted to the test session for 5 minutes and had their behavior recorded for later analysis.

For behavior evaluation, every 5 seconds of the test session, the predominant behavior of the animal was quantified, which can be passive behavior (immobility) or active behavior (swimming or climbing). The immobility behavior is characterized by the animal's absence of movement, except for some movements that allow it to float. Swimming behavior, in turn, is characterized by active movements of the animal in the cylinder, while climbing occurs when the animal moves towards the cylinder walls, indicating an attempt to escape. After the pre-test and test sessions, the animals were removed from the cylinder, dried with a towel, and returned to their respective cages. The water was changed between each session (Waltrick et al., 2022, Leão et al., 2022).

2.6. Evaluation of oxidative stress parameters

2.6.1. Sample (HIP, PFC and blood serum) preparation

Rats were euthanized through decapitation, and their prefrontal cortex (PFC), hippocampus (HIP) and serum were collected. The collected blood was immediately cooled and centrifuged 6000g for 20 minutes. Serum was used for ROS and protein analysis.

The dissected brain areas were then immediately frozen on dry ice and stored at -80 °C until further analysis. For analysis, brain samples were homogenized in a phosphate buffer (80nM, pH 6.5). The resulting homogenate was used to quantify the rate of lipid peroxidation (LPO), and total reactive oxygen species (ROS). A portion of the homogenate was subjected to centrifugation at 9000 g (VS-15000 CFNII, Vision Scientific, Daejeon, South Korea) for 20 minutes (Kanazawa et al., 2021; de Lima Silva et al., 2022).

2.6.2. Quantification of proteins

The quantification of total proteins was carried out using the Bradford method (Bradford et al.,1976). The absorbance of the reaction was measured at 595 nm using a microplate reader (BioTek Synergy HT, BioTek Instruments, Highland Park, VT, USA), with bovine serum albumin (BSA) used as the protein standard. Individual values were interpolated on a BSA standard curve (ranging from 125 to 1000 µg) and expressed in mg of protein (Lívero et al., 2016; Kanazawa et al., 2021).

2.6.3. Determination of the lipid peroxidation (LPO) levels

LPO levels were measured using the FOX-2 method as originally described by Jiang et al. (1991). For this, 100 µL of PFC and HIP supernatant were homogenized in 100 µL of methanol, followed by vortexing and centrifugation at 5000 rpm (VS-15000 CFNII, Vision Scientific, Daejeon, South Korea) for 5 minutes at 4°C. Subsequently, 100 µL of the resulting supernatant was mixed with 900 µL of FOX-2 reagent (Wolff's reagent; containing 4 mM BHT, 250 mM $FeSO_4$, 250 mM H_2SO_4, and 100 mM xylenol orange). The absorbance was measured at 560 nm using a microplate reader (BioTek Synergy HT, BioTek Instruments, Highland Park, VT, USA). Results were expressed as nmol/mg of protein (Kanazawa et al., 2021; de Lima Silva et al., 2022).

2.6.4. Total reactive oxygen species (ROS)

The total ROS content in the samples was quantified using the 2'7' dichlorofluorescein-diacetate (DCFH-DA) assay, following the method described by Driver et al. (2000). In brief, 200 µl of a 1:10 (in 0.1 M potassium phosphate buffer, pH 6.5) dilution of the supernatant of brains simple or serum was mixed with a DCFA solution (consisting of DCFA, ethanol, and dimethylsulfoxide) and incubated for 40 minutes at room temperature in the dark. The formation of dichlorofluorescein was then measured using a spectrofluorimeter. The results are expressed as fluorescence intensity (Zhong and Yin, 2014).

2.7. Experimental design

At the beginning, before inducing T1DM and at the end (after the final behavioral tests), the rats' body weight (BW) was measured to calculate weight gain (WG; by subtracting the BW on the last day of the experiment from the BW on day 0). Blood glucose (BG) levels were measured 72 hours after inducing experimental diabetes and again at the end of all experimental protocols.

During all experiments, a group of vehicle-treated non-diabetic rats (VEH/VEH) was included as a control for the diabetic condition. The rats were given one week to acclimate to the environment before starting the tests, and prior to each behavioral experiment, the rats underwent at least 30 minutes of habituation in the experimentation room. All behavioral testing was conducted between 1 PM and 5 PM.

Experiment 1 – It was conducted for evaluating the effect of PDX (at doses of 1, 3, and 10 ng/animal) on mechanical allodynia in rats with experimentally induced-T1DM (STZ). Prior to the induction of T1DM, a baseline measure was obtained by performing the electronic Von Frey test one day in advance on all rats. Five groups were formed (n = 8/group): VEH/VEH, STZ/VEH, STZ/PDX 1, STZ/PDX 3, and STZ/PDX 10. On the 14th day after inducing experimental T1DM, treatment with either VEH or PDX was initiated. Each rat received one intraperitoneal injection on days 14, 15, 18, 21, 24, and 27 of the experimental protocol. The electronic Von Frey test was conducted on days 14, 16, 18, 20, 22, 24, 26 and 28 of the protocol to evaluate the cumulative effect of PDX treatment on mechanical allodynia. On days

when injections and the electronic Von Frey test coincided, the treatment was administered after the measurement of mechanical allodynia to avoid acute effects. At the end of the protocol, the rats' BW and BG levels were measured before euthanasia by decapitation.

Experiment 2 – This experiment was conducted using independent groups to assess the effect of PDX treatment (1, 3, and 10 ng/animal) on depressive-like and anxiety-like behaviors in T1DM-induced (STZ) rats. Therefore, five groups were formed (n = 8/group): VEH/VEH, STZ/VEH, STZ/PDX 1, STZ/PDX 3, and STZ/PDX 10. The treatment followed the same protocol as in Experiment 1, with intraperitoneal injections administered on days 14, 15, 18, 21, 24, and 27. On the 26th day, the rats underwent the EPM test. On day 27th, the pre-test of mFST was performed, and the scheduled injection for that day was administered after the pre-test session. The following day, on the 28th day and at the conclusion of the protocol, the rats underwent OFT and shortly thereafter to the mFST. To complete the experiment, the rats' BW and BG levels were measured before euthanasia by decapitation.

Experiment 3 – Experiment 3 was performed to assess the effect of PDX (at doses of 1, 3, and 10 ng/animal) on oxidative stress parameters in rats with induced T1DM (STZ). This experiment was designed with independent groups of animals and performed by experimenters blinded to the experimental groups (DRR, KMO, AC). Once again, five groups were established for the experiment: VEH/VEH (n = 5-7), STZ/VEH (n = 5-7), STZ/PDX 1 (n = 5-7), STZ/PDX 3 (n = 5-7), and STZ/PDX 10 (n = 5-7). The treatment protocol followed the same approach as in Experiments 1 and 2, involving intraperitoneal injections administered on days 14, 15, 18, 21, 24, and 27. On the 28th day, the rats were euthanized by decapitation, and their brains were removed. The HIP and PFC of the rats were carefully dissected and stored in a freezer at – 80 ºC for subsequent analysis of the previously mentioned oxidative parameters. The blood samples were collected from the animals' trunk after decapitation into a heparinized tube to prevent coagulation before centrifugation for blood plasma separation.

2.8. Statistical analysis

The distribution profile of the data was assessed using the Shapiro-Wilk normality test to confirm a Gaussian curvature. Since the normality criteria were met, the data were presented as mean ± standard error of the mean (SEM). In Experiment 1, a two-way ANOVA with repeated measures was performed, with treatment and time as the factors. For Experiments 2 and 3, a Student's *t*-test was conducted to compare the VEH/VEH and STZ/VEH groups, followed by a one-way ANOVA among all the STZ groups, with the treatment (VEH or PDX) being the only independent factor. *Post-hoc* analysis was carried out using the Newman-Keuls test when applicable. Statistical significance was determined at a p-value < 0.05. All statistical analyses were performed using GraphPad Prism software (version 9, San Diego, CA, USA).

3. Results

3.1. Effect of prolonged PDX treatment on blood glucose levels and body weight in rats with experimentally induced T1DM

In relation to Experiment 1, the initial section of Table 1 presents the results for blood glucose (BG) and weight gain (WG). Regarding BG and WG, the Student's *t*-test confirmed the successful induction of T1DM, as there was a significant difference between the VEH/VEH and STZ/VEH groups [BG: t = 29.47, df = 13; p < 0.05; WG: t = 15.67, df = 13; p < 0.05]. Subsequent one-way ANOVA analysis conducted among the STZ groups revealed that treatment with PDX significantly improved BG in these animals, with statistically significant differences observed between the groups [F (3, 27) = 5.127; p < 0.05]. The Newman-Keuls *post-hoc* analysis further demonstrated that doses of 3 and 10 ng of PDX effectively improved BG levels in rats with induced T1DM. Regarding WG, the one-way ANOVA performed among the STZ groups showed significant differences between the evaluated groups [F (3, 27) = 4.859; p < 0.05]. Consistent with these findings, the Newman-Keuls test revealed that treatment with 3 and 10 ng doses of PDX significantly improved WG in STZ rats.

The data presented in the second section of Table 1 correspond to Experiment 2. Regarding to BG and WG, Student's *t*-test revealed a significant difference between the VEH/VEH and STZ/VEH groups [BG: t = 40.26, df = 14; p < 0.05; WG: t = 49.29, df = 14; p < 0.05], confirming the successful induction of

experimental T1DM in rats. Furthermore, the one-way ANOVA demonstrated a significant difference among the STZ groups treated with PDX compared to the STZ/VEH group on BG [$F(3, 28) = 5.171$; $p < 0.05$]. Subsequent Newman-Keuls *post-hoc* analysis indicated that PDX doses of 3 and 10 ng effectively lowered BG levels in rats with induced T1DM. Similarly, the one-way ANOVA demonstrated that treatment with PDX significantly improved the WG of STZ rats [$F(3, 28) = 8.034$; $p < 0.05$]. The Newman-Keuls test further indicated that the PDX doses of 3 and 10 ng were effective in enhancing the WG of rats with induced T1DM.

Table 1. Effect of treatment with vehicle (VEH; saline) or protectin DX (PDX; at doses 1, 3 and 10 ng/animal) on the parameters of blood glucose and weight gain normoclycemic and T1DM-induced rats.

Groups	Blood glucose (mg/dL)	Weight gain (g)
Experiment 1		
VEH/VEH	102.4 ± 1.82	169.6 ± 8.59
STZ/VEH	548.3 ± 16.15*	-13.57 ± 7.72*
STZ /PDX1	521.9 ± 18.77	3.12 ± 5.69
STZ /PDX3	488.3 ± 5.80#	24.75 ± 10.41#
STZ/PDX10	470.5 ± 16.72#	23.25 ± 7.73#
Experiment 2		
VEH/VEH	97.13 ± 2.46	52.25 ± 1.38
STZ /VEH	484.5 ± 9.30*	-29.75 ± 0.92*
STZ /PDX1	456.9 ± 17.45	-25.75 ± 2.25
STZ /PDX3	393.3 ± 29.2#	12 ± 1.47#
STZ /PDX10	417.4 ± 6.53#	20.38 ± 0.77#

Values were expressed as mean ± SD of 8 animals/experimental group. *$p < 0.05$ compared to VEH/VEH group; $^{\#}$ $p < 0.05$ compared to STZ/VEH.

3.2. Experiment 1

3.2.1. Prolonged treatment with PDX was able to alleviate mechanical allodynia in the electronic Von Frey test in rats with induced-T1DM

As depicted in Fig. 1, the two-way ANOVA with repeated measures revealed significant effects of the treatment factor [$F(4, 34) = 51.45$; $p < 0.05$], the time factor (days) [$F(7, 238) = 21.95$; $p < 0.05$], besides a significant interaction between these two factors [$F(28, 238) = 5{,}556$; $p < 0.05$]. The Newman-Keuls *post-hoc* analysis revealed a significant reduction in the mechanical threshold of the STZ groups compared to the VEH/VEH groups on the 14th day, indicating the development of mechanical allodynia in rats with experimentally induced T1DM. From day 16 onwards, the results remained consistent, and the doses of 3 and 10 ng/animal of PDX significantly increased the mechanical threshold of the rats compared to the STZ/VEH group. The VEH/VEH group did not exhibit significant changes in the mechanical threshold throughout the duration of the experiment (Fig. 1).

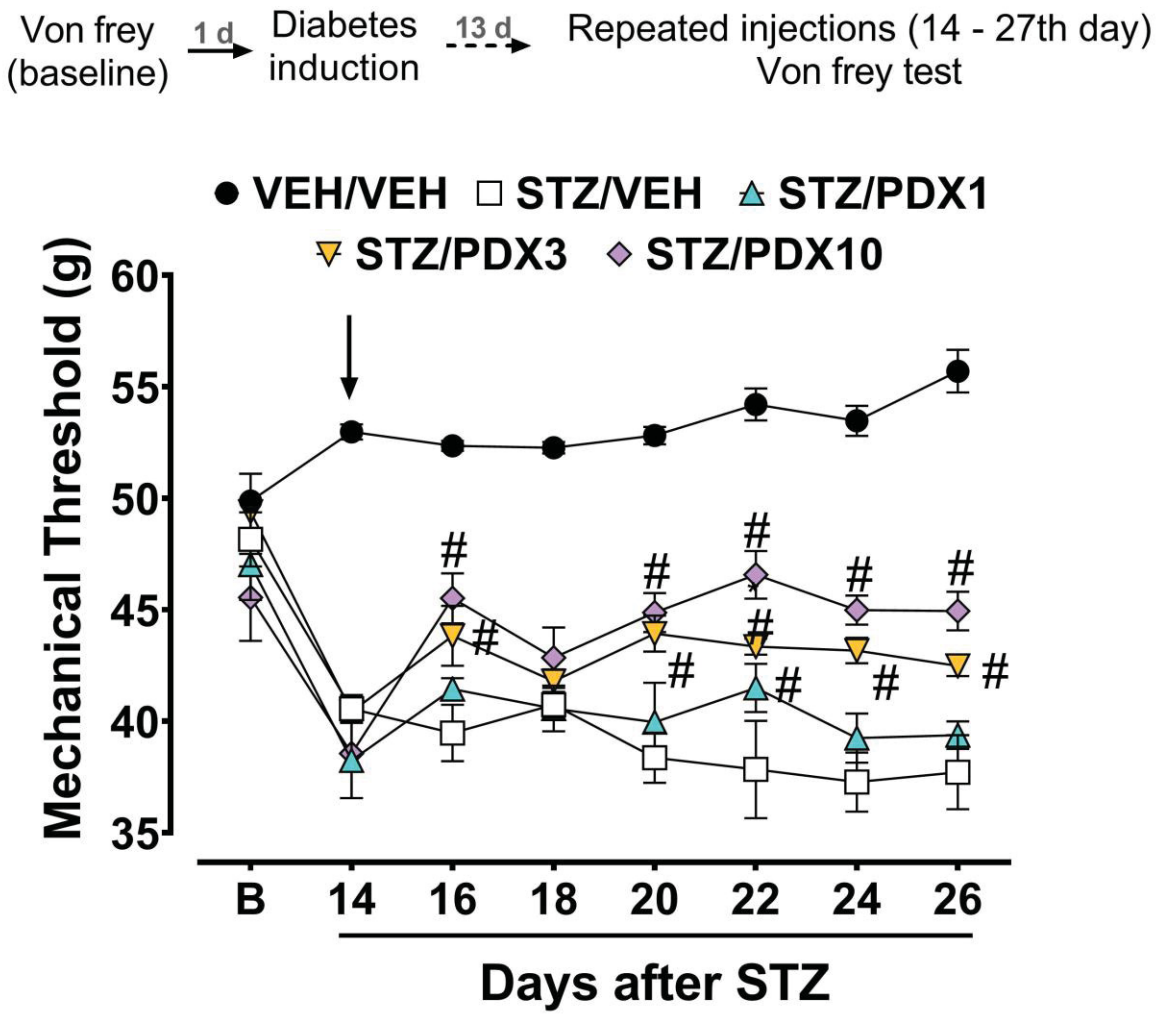

Fig. 1. Effect of the prolonged treatment with Protectin DX (PDX, 1, 3, or 10 ng/animal, i.p.) or vehicle (VEH, i.p.) on mechanical allodynia in experimental-T1DM (STZ) or normoglycemic (VEH/VEH) rats submitted to electronic Von Frey test. The injections took place on days 14, 15, 18, 21, 24, and 27 of the experimental protocol. Values were expressed as mean ± SEM (n = 8 rats/group). *p < 0.05 when compared to VEH/VEH; #p < 0.05 when compared to STZ/VEH. The arrow indicates the first day of treatment.

3.3. Experiment 2

3.3.1. Treatment with PDX significantly reduced the pronounced anxious-like behavior exhibited by T1DM-induced rats in the EPM test

The Student's *t*-test revealed significant differences between the VEH/VEH and STZ/VEH groups for all parameters assessed in the EPM test: percentage of time spent in open arms (Fig. 2, panel A) [t = 3.575, df = 14; p < 0.05], percentage of open arm entries (Fig. 2, panel B) [t = 4.091, df = 14; p < 0.05], number of entries into closed arms (Fig. 2, panel C) [t = 2.917, df = 14; p < 0.05], frequency of head dipping

(Fig. 2, panel D) [t = 2.917, df = 14; $p < 0.05$], and frequency of risk assessment (Fig. 2, panel E) [t = 3.121, df = 14; $p < 0.05$]. These findings confirmed the increased anxiety-like behavior observed in T1DM-induced rats (STZ/VEH) compared to normoglycemic rats (VEH/VEH).

In the one-way ANOVA analyses conducted on the STZ groups, significant differences were observed in the percentage of time and entries in the open arms (Fig. 2, panel A and B) [$F (3, 28) = 5.467$; $p < 0.05$ and $F (3, 28) = 6.888$; $p < 0.05$], as well as in the head dipping frequency (Fig. 2, panel D) [$F (3, 28) = 3.770$; $p < 0.05$]. The Newman-Keuls *post-hoc* analysis revealed that all three doses of PDX significantly improved the percentage of time spent in the open arms and the frequency of head dipping compared to the STZ/VEH group. Specifically for the percentage of entries into the open arms, the *post-hoc* analysis indicated that the PDX dose of 3 ng led to a significant improvement compared to the STZ/VEH group. On the other hand, the one-way ANOVA results did not show significant differences in entries into the closed arms (Fig. 2, panel C) [$F (3, 28) = 1.349$; $p < 0.05$] and the frequency of risk assessment (Fig. 2, panel E) [$F (3, 28) = 0.5128$; $p < 0.05$]. These findings suggest that PDX treatment effectively alleviated the heightened anxiety-like behavior observed in STZ-induced rats during the EPM test (Fig. 2).

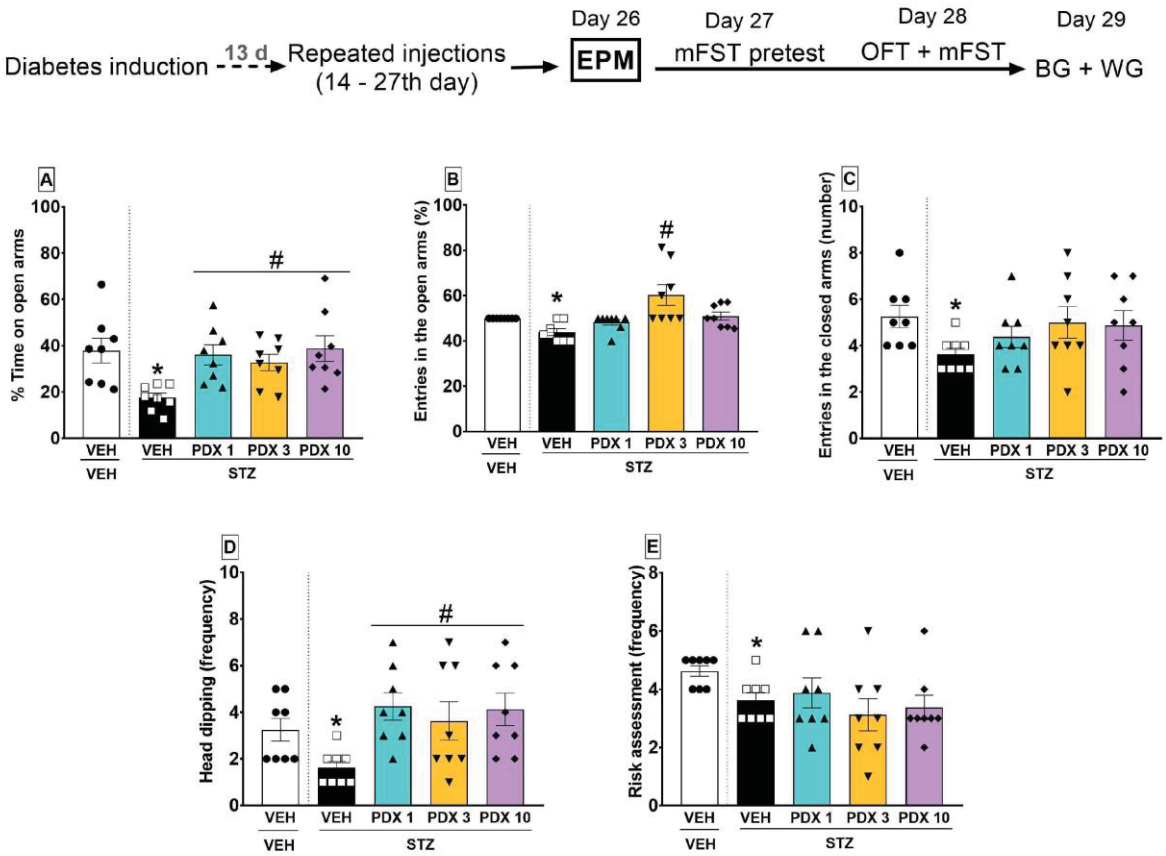

Fig. 2. Effect of the prolonged treatment with Protectin DX (PDX, 1, 3, or 10 ng/animal, i.p.) or vehicle (VEH, i.p.) on % time spent in the open arms (A), % frequency of open arms entries (B), number of enclosed arms entries (C), head dipping (D) and risk assessment (E) frequencies in experimental-T1DM (STZ) or normoglycemic (VEH/VEH) rats submitted to EPM test. The injections took place on days 14, 15, 18, 21, 24, and 27 of the experimental protocol. Values were expressed as mean ± SEM (n = 8 rats/group). *p < 0.05 when compared to VEH/VEH; #p < 0.05 when compared to STZ/VEH.

3.3.2. Treatment with PDX did not alter locomotor activity, but ameliorated anxiety-related parameters in the OFT in T1DM-induced rats

Student's *t*-test revealed significant differences in all three parameters assessed in the OFT between the STZ/VEH and VEH/VEH groups: number of crossings (Fig. 3, panel A) [t = 2.691, df = 14; p < 0.05], time spent in the center of the apparatus (Fig. 3, panel B) [t = 5.217, df = 14; p < 0.05], and rearing frequency (Fig. 3, panel C) [t = 3.710, df = 14; p < 0.05]. Furthermore, the one-way ANOVA applied to these parameters demonstrated a significant difference in time spent in the center (Fig. 3, panel B) [F (3, 28) = 4.77; p < 0.05] and rearing frequency (Fig. 3, panel C) [F (3, 28) = 5.178; p < 0.05], but not in the number of crossings (Fig. 3, panel A) [F (3, 28) = 1.043; p > 0.05]. The Newman-Keuls test revealed that the dose

of 3 and 10 ng of PDX significantly increased the time spent in the center of the open field and the frequency of rearing behaviors compared to the STZ/VEH group. These findings indicate that treatment with PDX did not affect locomotor activity but improved anxiety-related parameters in the OFT (Fig. 3).

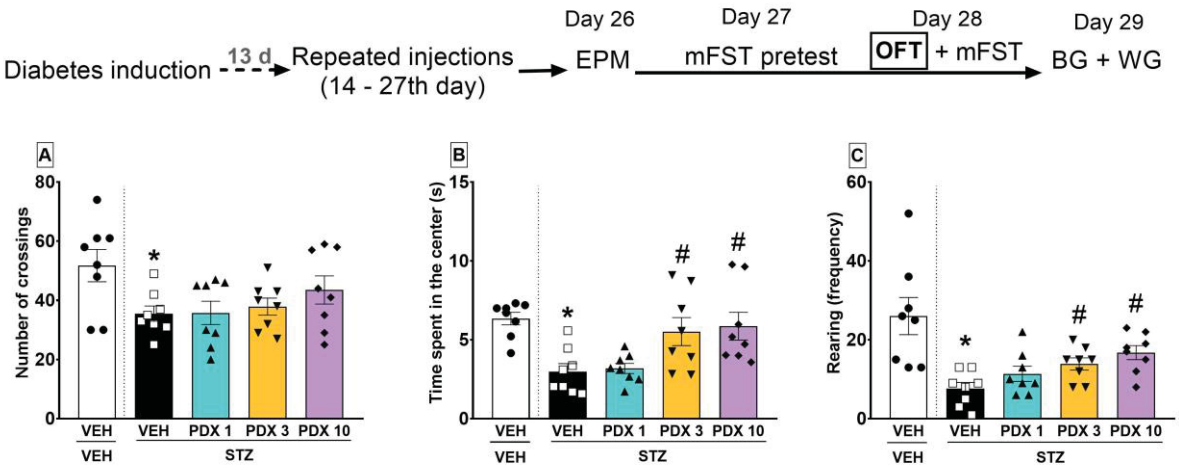

Fig. 3. Effect of the prolonged treatment with Protectin DX (PDX, 1, 3, or 10 ng/animal, i.p.) or vehicle (VEH, i.p.) on number of crossings (A), time spent in the center in seconds (B) and rearing frequency (C) in experimental-T1DM (STZ) or normoglycemic (VEH/VEH) rats submitted to OFT. The injections took place on days 14, 15, 18, 21, 24, and 27 of the experimental protocol. Values were expressed as mean ± SEM (n = 8 rats/group). *p < 0.05 when compared to VEH/VEH; #p < 0.05 when compared to STZ/VEH.

3.3.3. Prolonged treatment with PDX effectively ameliorated depressive-like responses in the mFST in T1DM-induced rats

The results obtained from the mFST analysis revealed significant differences between the STZ/VEH and VEH/VEH groups in all the parameters examined Student's *t*-test: frequency of immobility behavior (Fig. 4, panel A; t = 6.376, df = 14), frequency of swimming behavior (Fig. 4, panel B; t = 3.014, df = 14; p < 0.05), and frequency of climbing behavior (Fig. 4, panel C; t = 3.644, df = 14; p < 0.05). Subsequently, the one-way ANOVA analysis demonstrated significant differences between the PDX-treated groups and the STZ/VEH group in the following parameters: frequency of immobility behavior (Fig. 4, panel A; F (3, 28) = 14.02; p < 0.05) and frequency of swimming behavior (Fig. 4, panel B) [F (3, 28) = 14.98; p < 0.05]. Further analysis using the Newman-Keuls test revealed that all three doses of PDX significantly reduced the frequency of immobility and increased the frequency of

swimming compared to the STZ/VEH group. Notably, no statistical difference was observed between the STZ/PDX and STZ/VEH groups in terms of the frequency of climbing behavior (Fig. 4, panel C; F (3, 28) = 0.7632; p > 0.05). These data indicate that treatment with PDX was effective in ameliorating the prominent depressive-like behaviors observed in rats with experimentally induced T1DM during the mFST (Fig. 4).

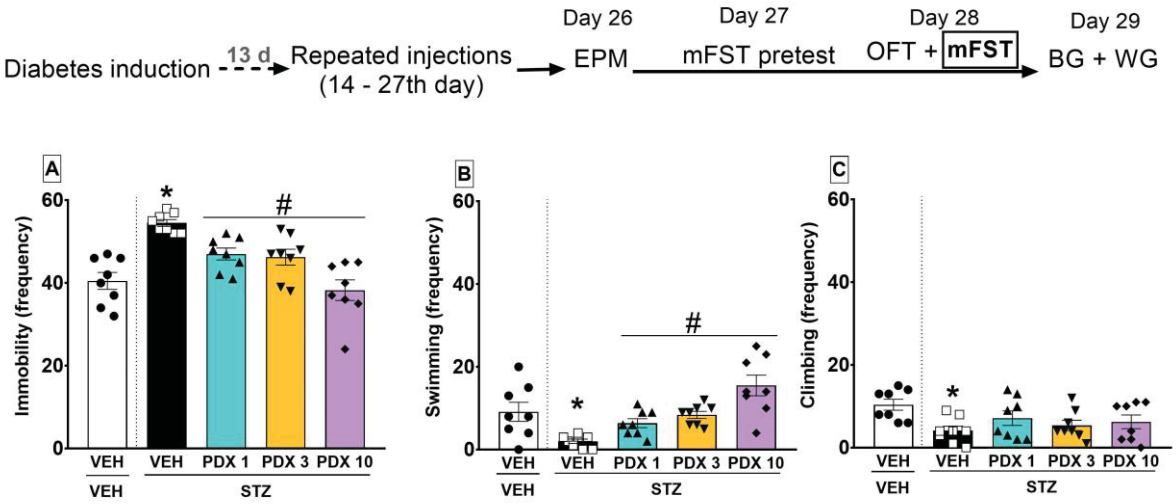

Fig. 4. Effect of the prolonged treatment with Protectin DX (PDX, 1, 3, or 10 ng/animal, i.p.) or vehicle (VEH, i.p.) on immobility frequency (A), swimming frequency (B), and climbing frequency (C) in experimental-T1DM (STZ) or normoglycemic (VEH/VEH) rats submitted to mFST. The injections took place on days 14, 15, 18, 21, 24, and 27 of the experimental protocol. Values were expressed as mean ± SEM (n = 8 rats/group). *p < 0.05 when compared to VEH/VEH; #p < 0.05 when compared to STZ/VEH.

3.4. Experiment 3

3.4.1. PDX treatment improved some oxidative stress parameters in T1DM-induced rats' PFC and HIP, but not all parameters analyzed

In Fig. 5, panels A and B (PFC and HIP, respectively) related to LPO, Student's *t*-test revealed significant differences between the VEH/VEH and STZ/VEH groups (Fig. 5, panels A and B) [t = 11.42, df = 8; p < 0.05 and t = 2.188, df = 9; p < 0.05]. This confirms increased lipid peroxidation in the analyzed brain structures of rats with induced T1DM compared to the normoglycemic group. One-way ANOVA demonstrated that PDX treatment reduced LPO levels in the PFC compared to the STZ/VEH group (Fig. 5, panel A) [F (3, 16) = 3.652; p < 0.05]. The Newman-Keuls *post-hoc* analysis revealed that the 10 ng dose of PDX significantly decreased LPO levels. Similarly, for the HIP, one-way ANOVA indicated that PDX treatment

effectively reduced LPO levels in this brain structure in rats with induced T1DM (Fig. 5, panel B) [$F(3, 20) = 7.532$; $p < 0.05$]. The Newman-Keuls test demonstrated that all three doses of PDX (1, 3, and 10 ng) significantly decreased LPO levels in the HIP.

Regarding the results of ROS levels in the PFC and HIP showed significant differences in the Student's *t*-test, indicating an increase in ROS levels in the STZ/VEH group compared to the VEH/VEH group (Fig. 5, panels C and D, respectively,$t = 2.712$, $df = 8$; $p < 0.05$ and $t = 3.111$, $df = 8$; $p < 0.05$]. One-way ANOVA revealed differences between the groups treated with PDX and the STZ/VEH group only in the HIP (Fig. 5, panel D; $F(3, 16) = 4.279$; $p < 0.05$). The Newman-Keuls test demonstrated that all three doses of PDX (1, 3, and 10 ng) significantly decreased ROS levels in the HIP.

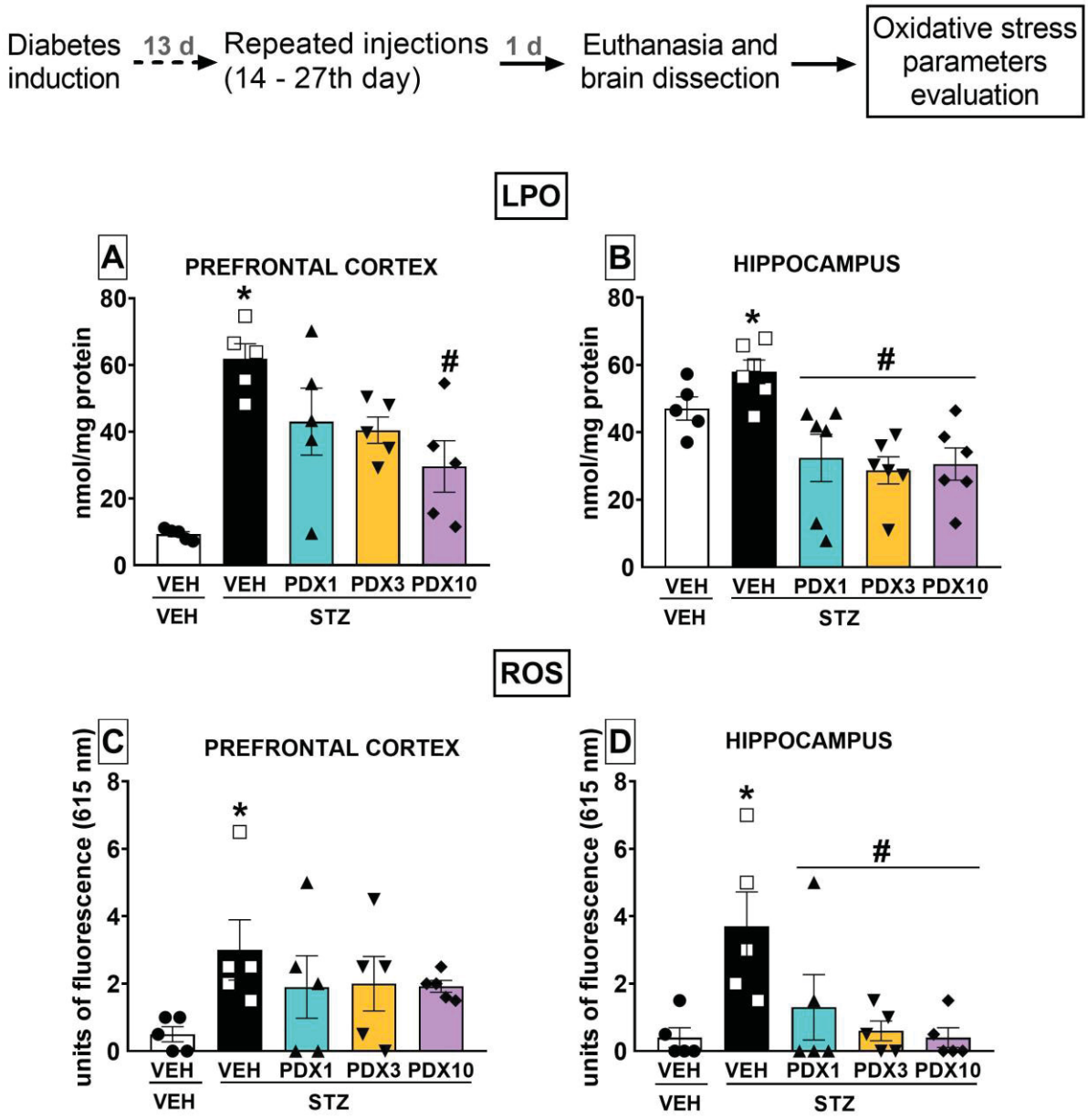

Fig. 5. Effect of the prolonged treatment with Protectin DX (PDX, 1, 3, or 10 ng/animal, i.p.) or vehicle (VEH, i.p.) in experimental-T1DM (STZ) or normoglycemic (VEH/VEH) rats on lipid peroxidation levels (LPO, panel A – prefrontal cortex, panel B – hippocampus), and total reactive oxygen species (ROS, panel C – prefrontal cortex, panel D – hippocampus). The injections took place on days 14, 15, 18, 21, 24, and 27 of the experimental protocol. Values were expressed as mean ± SEM (n = 5-7 rats/group). *p < 0.05 when compared to VEH/VEH; #p < 0.05 when compared to STZ/VEH.

3.4.2. Treatment with PDX successfully reduced ROS levels in the serum samples of rats with induced T1DM

The results of the Student's *t*-test revealed significant differences in serum ROS levels between the STZ/VEH and VEH/VEH groups (Fig. 6) [t = 2.322, df = 8; p < 0.05]. One-way ANOVA showed statistical differences between the STZ/VEH and STZ groups treated with PDX [F (3, 16) = 6.341; p < 0.05]. The Newman-Keuls *post-hoc* analysis demonstrated that all three doses of PDX (1, 3, and 10 ng) effectively reduced ROS levels in the serum of rats with induced T1DM (Fig. 6).

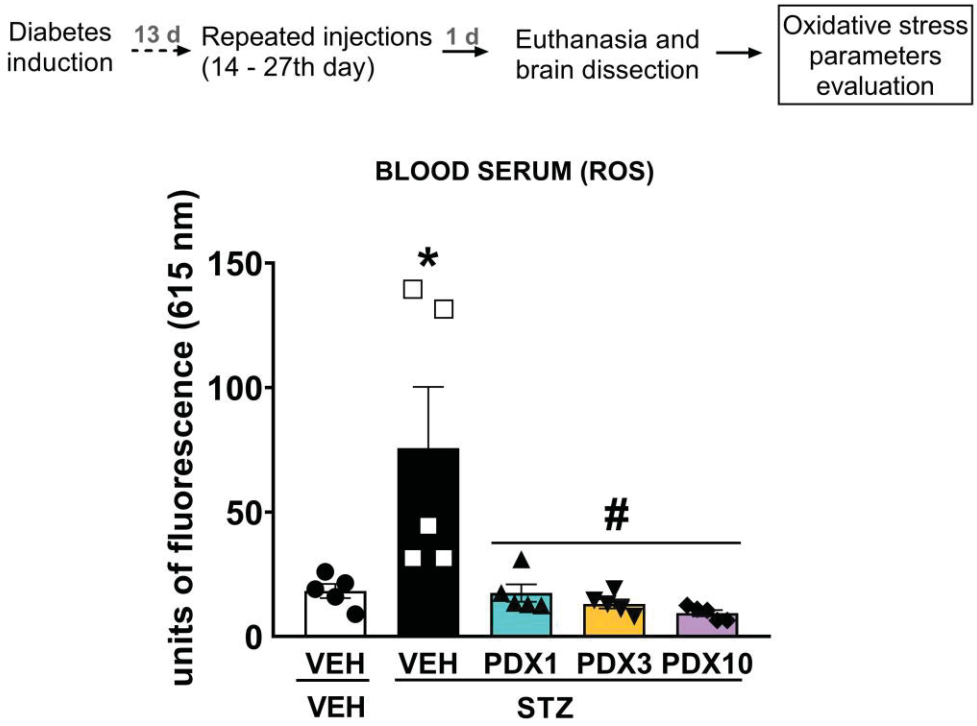

Fig. 6. Effect of the prolonged treatment with Protectin DX (PDX, 1, 3, or 10 ng/animal, i.p.) or vehicle (VEH, i.p.) in experimental-T1DM (STZ) or normoglycemic (VEH/VEH) rats on total reactive oxygen species (ROS, serum from blood samples). The injections took place on days 14, 15, 18, 21, 24, and 27 of the experimental protocol. Values were expressed as mean ± SEM (n = 5-7 rats/group). *p < 0.05 when compared to VEH/VEH; #p < 0.05 when compared to STZ/VEH.

4. Discussion

The major findings of this study reveal that continuous PDX treatment, across all tested doses, ameliorates mechanical allodynia and prevents anxiety-like behaviors, and depressive-like behaviors in STZ-induced T1DM rats. These positive outcomes may be, in part, linked to its neuroprotective and antioxidant properties, as evidenced by PDX-treatment significant mitigation of total ROS and LPO elevation in the HIP, PFC, and total ROS in the serum of STZ rats. It is noteworthy that this is the first study to assess the potential beneficial effects of PDX in treating comorbidities associated with T1DM in an animal model. Interestingly, PDX treatment effectively improved some diabetic parameters by reducing glycemic levels and countering the reduced weight gain characteristic of the T1DM animal model.

STZ is a DNA alkylating antibiotic widely employed to induce the T1DM animal model. Through a single injection of STZ, this agent significantly diminishes the population of insulin-producing pancreatic β cells, resulting in the characteristic hyperglycemic state of T1DM (Wu and Yan, 2015). Furthermore, this animal model of T1DM exhibits other important disease-related features such as reduced weight gain and an inflammatory profile, along with behavioral changes that emerge during the experimental protocols (Chaves et al., 2021; de Lima Silva et al., 2022; Leão et al., 2022; Waltrick et al. 2023). As expected, the present study was able to replicate the experimental model of T1DM induced by STZ, i.e., rats administered with STZ exhibited elevated blood glucose levels and reduced weight gain, along with a decrease in the mechanical threshold associated with neuropathic pain, as well as depressive-like and anxious-like behaviors (de Morais et al., 2014, 2018; Redivo et al., 2016; Jesus et al., 2019; Gasparin et al., 2021; Chaves et al., 2021; Leão et al., 2022).

As observed in Table 1, in the two conducted experiments, PDX at the two highest doses (3 and 10 ng/animal) ameliorated parameters associated with the diabetic condition, i.e., reduced the hyperglycemia levels and improved the weight gain from these T1DM rats. Regarding the diabetic condition, there are limited studies on the effects of PDX in both T1DM and DMT2 mouse models. It has been demonstrated that PDX can reduce insulin resistance in a DMT2 model, lower fasting blood glucose levels in both T1DM and DMT2 models and improving weight gain in

mice (White et al., 2014; Rengachar et al., 2023). Interestingly, in a recent study from our group, we also demonstrated an improvement in the same diabetic parameters in T1DM rats; however, after the treatment with other SPM derived from DHA, the resolvin-D5 (Leão et al., 2022).

Our data from the electronic Von Frey test align with findings from other studies, indicating an increase in mechanical allodynia in STZ/VEH rats starting from the 14th day after T1DM induction, as demonstrated by a decrease in the mechanical threshold during the test. The rise in mechanical allodynia progressively intensifies throughout the protocol, reaching its peak on the 26th day (Redivo et al., 2016, 2019; Jesus et al., 2019; Gasparin et al., 2021). Starting from the second day of PDX treatment, an increase in the mechanical threshold was observed in STZ rats treated with doses of 3 and 10 ng/animal. This improvement in mechanical allodynia became consistent from the third injection and continued throughout the protocol. In a 2022 study, Zhao and colleagues demonstrated in a lumbar radicular pain (LRP) model in male Sprague-Dawley rats that intrathecal treatment with PDX at doses of 10 and 100 ng for 3 days increased the mechanical threshold in the electronic Von Frey test, as well as thermal withdrawal latency, compared to the control group (Zhao et al., 2022). The authors propose that the antinociceptive effect of PDX could be linked to the suppression of pro-inflammatory cytokine mRNA, including IL-6 and IL-1β, and the facilitation of inflammation resolution via the autophagy flux mechanism. This mechanism has been recognized as significant in alleviating specific types of pain, such as neuropathic pain (Weng et al., 2019; Zhao et al., 2022).

Other studies have reported beneficial effects of PDX and its PD1 isomer on various types of pain, including inflammatory pain in rat models induced by carrageenan and DNP in mice (Xu et al., 2013; Fonseca et al., 2017; Nesman et al., 2021). Regarding other D-series SPMs, several studies have been conducted using various models of inflammatory and neuropathic pain. Treatments with RvD1 and RvD5, as well as MaR 1, have demonstrated analgesic effects (Ji et al., 2011; Fattori et al., 2019; Wang et al., 2022). However, the precise mechanisms underlying these effects are still inconclusive, although some evidence suggests a reduction in components associated with pain-related inflammation. The precursor of these SPMs, PUFA n-3 DHA, has been extensively studied in the context of neuropathic pain in animal models of experimental T1DM (Redivo et al., 2016, 2019). The mechanisms proposed to explain these effects, in addition to the well-documented

anti-inflammatory action of n-3 PUFAs like DHA, include its neuroprotective properties, possibly through the upregulation of neurotrophins like BDNF, and a potential involvement of the opioid system (Redivo et al., 2016, 2019).

As mentioned previously, this study is the first to assess the impact of PDX treatment on DNP associated to anxiety-like and depressive-like behavior. Nevertheless, there are some studies in the literature that have examined these behaviors using other D-series SPMs, as well as with the precursor of SPMs, DHA. Regarding anxiety-like behavior, our findings in the EPM test and the OFT align with previous studies conducted by our group and other researchers, all of which indicate a more pronounced anxious-like behavior in the STZ/VEH group compared to normoglycemic rats (VEH/VEH) (Chaves et al., 2021; Leão et al., 2022; Waltrick et al., 2023; Lu et al., 2023; Vasović et al., 2023). In the EPM test, the treatment with all three doses of PDX prevented the more pronounced anxiety-like response by significantly increasing the time spent in the open arms of the apparatus from STZ-induced diabetic rats, along with an increase in the frequency of head dipping. However, when it comes to the number of entries into the open or closed arms, only the dose of 3 ng of PDX displayed a notable increase in the entry into open arms, indicating an anxiolytic-like effect, while there is no difference in the number of entries into the closed arms.

The frequency of head dipping is lower in animals displaying anxious-like behavior since they tend to avoid situations that may pose a potential threat, which is represented here by the open arms of the EPM (Silva and Brandão, 2000). In a study conducted by our laboratory, assessing the therapeutic potential of RvD5 in STZ-induced T1DM animals, we observed comparable outcomes in the EPM tests, except for risk assessment (Leão et al., 2022). Risk assessment is an ethological measure assessed in certain fear and anxiety-related behavioral tests. It is considered a pattern of defensive behavior that occurs when rodents perceive a potential threat (Blanchard et al., 1993; for a review, see Carobrez and Bertoglio, 2005). This behavior has been assessed in studies conducted by our research group in rats with STZ-induced T1DM, yielding some inconclusive results (Waltrick et al., 2022; Leão et al., 2022).

As for DHA, the precursor of PDX, recent studies have demonstrated an anxiolytic-like effect in various animal models of anxiety (Pérez et al., 2013; Laugero et al., 2017; Neto et al., 2022). Neto and colleagues investigated the impact of EPA

and DHA supplementation on various parameters in a rat model of obesity induced by a high-calorie diet. This supplementation was found to effectively reduce anxiety-like behaviors in animals during EPM test. Furthermore, the supplementation led to a reduction in inflammatory markers within the PFC of obese rats, such as TNF-α, attributed to the anti-inflammatory properties of these compounds (Neto et al., 2022). Additionally, it's worth noting that animals on a DHA-deficient diet exhibit more pronounced anxiety-like behavior in comparison to rodents on a standard diet (Bhatia et al., 2011).

In the OF test, STZ/VEH rats exhibited a reduction in the number of crossings within the apparatus, consistent with previous findings (Redivo et al., 2016; de Morais et al., 2018; Waltrick et al., 2022). PDX treatment did not alter this behavior in rats. The decrease in the number of crossings is indicative of reduced exploratory activity in STZ rats, as supported by the decreased frequency of rearing, another exploratory behavior. Notably, doses of 3 and 10 ng of PDX were effective in increasing the frequency of rearing. Furthermore, the time spent in the center of the apparatus, which is diminished in STZ/VEH rats, indicative of anxiety-like behavior, was restored in treated animals (3 and 10 ng of PDX), when compared to VEH/VEH rats.

In a study involving MaR1, a SPM also derived from DHA, a single injection (5 μg/kg via i.p.) was able to ameliorate anxiety-like behaviors observed in the OFT. These behaviors included the time and frequency of entries into the center of the apparatus within a lipopolysaccharide (LPS)-induced depression-like model in mice (Shi et al., 2023). However, it's noteworthy that this single injection, differently of our study, did not completely reverse these behaviors but did provide relief. However, even though it is one single administration, MaR 1 proved be effective in reducing heightened microglial activation observed in different markers, such as the assessment of the Iba-1 biomarker in the HIP of mice and the reduction in mRNA expression of inflammatory factors (Shi et al., 2023). Like its preventive effects on anxiety-like behaviors in STZ rats, PDX also demonstrated significant improvements on more pronounced depressive-like behaviors from STZ-diabetic animals in the mFST. All three doses of PDX were enough to prevent the depressive-like behavior from STZ-animals by decreasing the frequency of passive behavior (immobility) and increasing active behavior (swimming). Once more, it's worth noting that this study marks the first evaluation of PDX's impact on depressive-like behaviors. However, other SPMs have been investigated as potential therapeutic options for depression

(for a review, see Giacobbe et al., 2020). Most studies investigating the antidepressant-like effects of SPMs have focused on RvDs, specifically RvD1, RvD2, and RvD5 (Deyama et al., 2017; Leão et al., 2022). In an inflammatory depression model induced by LPS in mice, Deyama and colleagues observed the antidepressant-like effects of RvD1 and RvD2, both administered at a dose of 10 ng, as evidenced in the tail suspension test (TST). Interestingly, when Rapamycin, an mTORC1 inhibitor, was combined with RvDs, the antidepressant-like effects were abolished, suggesting an association with the mTORC1 signaling pathway. This is similar to the mechanism of action of fast-acting antidepressants like ketamine (Deyama et al., 2017).

Regarding the treatment with SPMs of T1DM animals and its action on depressive-like behaviors, our research group is the only one that examined the effects of RvD5 on behaviors related to depression (Leão et al., 2022). However, unlike the present study, RvD5 was able to improve depressive-like behavior and not restore or reestablish behavior to levels comparable to non-diabetic animals. Thus, Leão and coworkers demonstrated that RvD5 reduced the frequency of immobility and increased the frequency of swimming in the mFST (Leão et al., 2022). Interestingly and like what we observed with PDX, RvD5 was able to alter only swimming and not climbing frequency. It has been demonstrated that an increase in swimming frequency has been linked to enhanced serotonergic neurotransmission (Cryan et al., 2002; Slattery and Cryan, 2012).

Furthermore, investigations involving DHA, the n-3 PUFA precursor shared by PDX and RvD, have previously demonstrated that dietary supplementation with this compound, either directly or indirectly, promotes increased serotonin and its metabolite levels in the brains of rodents (Redivo et al., 2016; Waltrick et al., 2022). Thus, an increase in serotonin levels in brain areas important for mediating and modulating emotions, as well as pain, may be involved in this beneficial effect of compounds derived from DHA as RvD5 and PDX on these behaviors. It is worth noting that although in the present study PDX demonstrated a preventive effect for depressive and anxiogenic-like behaviors, the same was not observed for DNP, which only had a significant improvement but not prevention of the painful condition. This difference may arise from the route of administration, which may interfere with the bioavailability, and doses of PDX. In Zhao and colleagues' study (2022), the mechanical threshold in the LRP model in rats was assessed after intrathecal

injection of PDX at two doses (10 ng and 100 ng), a local route related to analgesic effects. Although the 100 ng dose did not completely reverse mechanical allodynia, it came closer to the control group. The authors discuss the resolution mechanism through autophagy flux, which occurs in a dose-dependent manner (Zhao et al., 2022). Therefore, it is possible that treatment with PDX via systemic intraperitoneal injections, even at the 10 ng dose, may not have reached the full resolution potential of this SPM regarding pain-related behaviors, maybe due to a decrease in bioavailability.

Following the observation of behavioral alterations in rats with experimentally induced T1DM that were either prevented (anxiety and depression) or improved DNP by PDX, the last study aimed to explore whether PDX would exert a protective action through of antioxidant properties. It is unsurprising that STZ/VEH rats exhibited elevated levels of reactive oxygen species ROS in both the brain and serum, along with increased LPO in the brain as well (HIP and PFC for both measures). Numerous studies, including those conducted by our laboratory and others, have consistently highlighted oxidative stress as a significant contributor to T1DM (Giacco and Brownlee, 2010; de Morais et al., 2014; Pereira et al., 2018; Gasparin et al., 2021; de Lima Silva et al., 2022) and its comorbidities.

Our data demonstrated that HIP was more sensitive to the protective effects of PDX when compared to PFC. For example, in HIP, in all doses PDX was able to prevent the LPO and ROS high levels, while in the PFC, only the 10 ng dose of PDX exhibited a significant reduction in LPO levels, with no significant alteration in ROS levels. However, the levels of ROS in the serum (blood plasma), which were elevated in STZ/VEH rats, were effectively prevented by the administration of PDX in all three doses assessed. It is known that brain tissue has a lower antioxidant capacity due to a series of factors, such as high oxidative metabolic activity, high production of ROS, and low levels of antioxidant agents (Lee et al., 2020). The diabetic brain is affected by oxidative stress, and it is possible that this difference in the antioxidant action of PDX in the HIP compared to the PFC occurs due to the difference in oxidative stress levels in these regions (de Morais et al., 2014). For instance, Muriach and colleagues point out the hippocampus as a key structure in relation to oxidative stress in diabetes, with high levels of oxidant enzymes and low levels of antioxidant enzymes, which may make it more influenced by antioxidant agents (Muriach et al., 2014).

In contrast to the few results involving behavior related to pain and emotion and the effects of compounds derived from DHA, there exists a body of literature detailing the antioxidant properties of PDX. These studies encompass various animal models in both rats and mice, as well as *in vitro* experiments (Liu et al., 2014; Hwang et al., 2019; Zhao et al., 2022; Rengachar et al., 2023). Rengachar and colleagues demonstrated that treatment with PDX (50 ng/animal, i.p.) in mice with experimental T1DM and DMT2 was able to reduce markers of oxidative stress in the animals' plasma and pancreas, including LPO levels (Rengachar et al., 2023).

Other SPMs have been studied to understand their antioxidant action since oxidative stress and inflammatory processes are closely intertwined, and SPMs primarily function to resolve inflammation (for a review, see Wang et al., 2020). NPD1, an isomer of PDX, has also shown effectiveness in regulating proteins related to apoptosis in *in vitro* experiments under oxidative stress conditions. This includes upregulating antiapoptotic proteins such as Bcl-2 and Bcl-xL, while downregulating proapoptotic proteins like Bad and Bax (Mukherjee et al., 2004; Bazan et al., 2011). These mechanisms warrant further exploration to elucidate the antioxidant action of protectins and other SPMs. The specialized literature also highlights the antioxidant properties of fish oil, which is rich in n-3 PUFAs like EPA and DHA, both precursors of different SPMs (Staziaki et al., 2013; Asari et al., 2021). In addition to their role in regulating antioxidant enzymes, n-3 PUFA supplementation has shown the capacity to reduce ROS and LPO levels, a characteristic shared with PDX in the current study.

Taken together, our findings demonstrate that PDX induces relief on mechanical allodynia and mitigates anxiety-like and depressive-like behaviors in rats with experimentally induced-T1DM using STZ. A plausible mechanism underlying these effects are the potential antioxidant properties of PDX, as evidenced by reduced levels of ROS and LPO in key brain regions involved in emotional regulation, including the PFC and/or HIP, as well as in the blood plasma of the animals. Therefore, it is important to delve deeper into studies to better understand the mechanisms related to the therapeutic potential of PDX for managing T1DM-associated comorbidities, highlighting once again the promising prospects of SPMs as protective therapeutic agents to treat these comorbidities and conditions directly linked to T1DM.

Author contributions

Janaina Menezes Zanoveli, Joice Maria da Cunha and Waldiceu Aparecido Verri Jr were responsible for the conception and design of the study. The study methodology was developed and validated by Janaina Menezes Zanoveli, Joice Maria da Cunha and Alexandra Acco. The preparation of the material and data collection were carried out by Ana Paula Farias Waltrick, Fábio Augusto Antunes Brito, Débora Rasec Radulski and Kauê Marcel de Oliveira. Statistical analysis was carried out by Ana Paula Farias Waltrick and Janaina Menezes Zanoveli. The first version of the manuscript was written by Ana Paula Farias Waltrick and Janaina Menezes Zanoveli. All authors read and approved the final manuscript.

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Declarations of interest

The authors declare no conflicting interests.

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4 CONSIDERAÇÕES FINAIS

Os resultados do nosso estudo demonstraram que a Protectina DX é capaz de reduzir a dor neuropática diabética e prevenir comportamentos tipo-ansiosos e tipo-depressivos em ratos com DMT1 induzido experimentalmente pela estreptozotocina. Apesar da necessidade de estudos para elucidar mecanismos de ação, nossos achados ressaltam a importância de propriedades protetoras do PDX em induzir essas ações benéficas sobre comorbidades do DMT1; demonstradas no estudo através da ação antioxidante no HIP, CPF e no soro sanguíneo. Cabe ainda ressaltar, que o tratamento melhorou parâmetros da própria condição diabética, como o ganho do peso e os níveis glicêmicos, o que impacta positivamente o bem-estar e sobrevida dos animais.

Em conclusão, nossos achados ressaltam que, por ser o DMT1 uma doença com característica inflamatória, a busca por compostos com perfil protetor (ação antioxidante e anti-inflamatória) torna-se urgente. Assim, os compostos MLPR devem ser mais bem explorados pelas perspectivas promissoras como agentes terapêuticos para tratar essas comorbidades e condições diretamente ligadas ao DMT1.

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