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HELEN DE MORAIS

AUMENTO DO ESTRESSE OXIDATIVO NO HIPOCAMPO E NO CÓRTEX PRÉ-FRONTAL ESTÁ RELACIONADO AO COMPORTAMENTO DO TIPO DEPRESSIVO EM RATOS DIABÉTICOS POR ESTREPTOZOTOCINA

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Dissertação apresentada ao Programa de Pós-Graduação em Farmacologia da Universidade Federal do Paraná como requisito parcial para obtenção do título de Mestre em Farmacologia.

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PARECER

A Comissão Examinadora da Dissertação de Mestrado intitulada "AUMENTO DO ESTRESSE OXIDATIVO NO HIPOCAMPO E NO CÓRTEX PRÉ-FRONTAL ESTÁ RELACIONADO AO COMPORTAMENTO DO TIPO DEPRESSIVO EM RATOS DIABÉTICOS POR ESTREPTOZOTOCINA", de autoria da pós-graduanda **HELEN DE MORAIS,** sob orientação da Prof.ª Dr.ª Janaína Menezes Zanoveli e composta pelos professores: Prof.ª Dr.ª Janaína Menezes Zanoveli (Presidente – Farmacologia – UFPR); Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Farmacologia – UFPR) e Prof.ª Dr.ª Anete Curte Ferraz (Fisiologia – UFPR), reuniu-se e, de acordo com o Regimento Interno do Programa de Pós-Graduação em Farmacologia, a pós-graduanda foi APROVADA. Para a devida publicação o trabalho deverá sofrer as modificações sugeridas, que serão conferidas por sua orientadora. Em Curitiba, 20 de setembro de 2013.

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Dedico este trabalho aos meus pais, pelo exemplo,

dedicação, apoio e amor fortalecedor.

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“A solução de problemas só restaura a normalidade.

As oportunidades significam explorar novos caminhos”
PETER DRUCKER

NOTA EXPLICATIVA

Esta dissertação é apresentada em formato alternativo, como artigo científico para publicação, de acordo com as normas do Programa de Pós-Graduação em Farmacologia da Universidade Federal do Paraná. A dissertação consta de uma revisão bibliográfica e hipótese do trabalho e um artigo científico com os experimentos realizados, resultados e discussão, além da conclusão sintetizando os achados do mesmo.

RESUMO

A depressão é uma comorbidade comum em pacientes diabéticos. Os mecanismos fisiopatológicos que relacionam esta comorbidade não estão completamente elucidados, embora as evidências apontem que o aumento do estresse oxidativo resultante da hiperglicemia pode ter um papel crucial. Assim, o efeito do tratamento prolongado com insulina (INS), o antioxidante da vitamina E (Vit E) ou com o antidepressivo imipramina (IMI), foi avaliado sobre resposta comportamental relacionada com a depressão e sobre parâmetros de stress oxidativo (níveis de produtos de peroxidação lipídica, níveis de glutationa reduzida e atividades da superóxido dismutase e catalase) em duas regiões encefálicas relacionadas com a depressão, o córtex pré-frontal (CPF) e o hipocampo (HIP). Nossos dados mostram que o tratamento com INS (6 UI / dia, sc) de ratos com o diabetes induzido experimentalmente pela estreptozotocina (ratos diabéticos –DBT) impediu o aumento de glicose no sangue, reduziu o tempo de imobilidade, um comportamento do tipo antidepressivo, e normalizou a redução do ganho de peso observada em animais DBT. Embora o tratamento com VIT E (300 mg / kg, vo) não tenha alterado os níveis de glicose no sangue, o tratamento foi capaz de reduzir o tempo de imobilidade e restabelecer o reduzido ganho de peso em ratos DBT. Diferentemente, o tratamento com IMI (15 mg / kg, ip) induziu comportamento do tipo antidepressivo também em animais normoglicêmicos além dos animais DBT. Enquanto o tratamento com VIT E e IMI restaurou apenas alguns parâmetros específicos do estresse oxidativo, o tratamento com INS foi capaz de prevenir todos os parâmetros alterados avaliados no CPF e no HIP de animais DBT. Assim, nossos dados fornecem provas adicionais da importância do envolvimento do estresse oxidativo no CPF e HIP na fisiopatologia da depressão relacionada ao diabetes.

Palavras chaves: diabetes, depressão, estreses oxidativo, insulina, vitamina E e imipramina.

ABSTRACT

Depression is a common comorbid in diabetic patients. The pathophysiologic mechanisms that relate this comorbidity is not completely elucidated yet, although evidences point out that increased oxidative stress resulting from hyperglycemia may have a crucial role. Thus, the effect of prolonged treatment with insulin (INS), the antioxidant vitamin E (VIT E) or the antidepressant imipramine (IMI) was evaluated on behavioral response related to depression and on oxidative stress parameters (lipid peroxidation product levels, reduced gluthatione levels and catalase and superoxide dismutase activities) in prefrontal cortex (PFC) and hippocampus (HIP). Our data show that treatment of streptozotocin-induced diabetic (DBT) rats with INS (6 UI/day, s.c.) prevented the blood glucose increase, reduced the immobility time, an antidepressant-like behavior, and normalized the reduced weight gain. Although the VIT E treatment (300 mg/kg, p.o.) had not altered the blood glucose levels, this treatment was able to reduce the immobility time and to reestablish the reduced weight gain in DBT rats. Differently, treatment with IMI (15 mg/kg, i.p.) induced antidepressant-like behavior in normoglycemic besides DBT animals. While VIT E and IMI treatments restored only specific oxidative stress parameters, INS was able to prevent all changed parameters evaluated in both PFC and HIP from DBT animals. Therefore, our data provide further evidence of the importance of oxidative stress in PFC and HIP in the pathophysiology of depression related to diabetes.

Keywords: diabetes, depression, oxidative stress, vitamin E, insulin, imipramine

LISTA DE ABREVIATURAS

AGEs – Produtos Finais de Glicação Avançada

ATP – Adenosina Trifosfato

CAT – Catalase

CPF – Córtex Pré-Frontal

DM – *Diabetes Mellitus*

DM1 – *Diabetes Mellitus* tipo 1

DM2 – *Diabetes Mellitus* tipo 2

DNA – Ácido Desoxirribonucleico

ERN – Espécie Reativa de Nitrogênio

ERO – Espécie Reativa de Oxigênio

GLUT2 – Transportador de Glicose tipo 2

GPX – Glutationa Peroxidase

GSH – Glutationa Reduzida

GSSG – Glutationa Oxidada

HbA – Hemoglobina A

HbA1 – Hemoglobina Glicada

HIP – Hipocampo

HPA – Hipotálamo-Pituitária-Adrenal

IMI – Imipramina

INS – Insulina

LPO – Peroxidação Lipídica

NAD – Nicotinamida Adenina Dinucleotídeo

PKC – Proteína Quinase C

SBD – Sociedade Brasileira de Diabetes

SOD - Superóxido Dismutase

STZ – Estreptozotocina

TNF – Teste de Natação Forçada

VIT E – Vitamina E

SUMÁRIO

1.0. REVISÃO LITERÁRIA

1.1. DIABETES

Diabetes Mellitus (DM) é um distúrbio metabólico de etiologia múltipla caracterizada pela hiperglicemia resultante da falha na ação ou secreção de insulina ou ambos, resultando em deficiência relativa ou absoluta da insulina (BROWNLEE, 2005; XIANG et al., 2010; ASSOCIAÇÃO AMERICANA DO DIABETES (ADA), 2009). De acordo com a ORGANIZAÇÃO MUNDIAL DE SAÚDE (2012), os danos causados pelo DM incluem danos a longo prazo como disfunção e falha de vários órgãos.

Segundo a FEDERAÇÃO INTERNACIONAL DO DIABETES (2012), estima-se que existam mais de 371 milhões de pessoas portadoras de DM. Mais ainda, esse número passaria para 552 milhões de pessoas até o ano de 2030. Desses 371 milhões de pessoas com DM, apenas 185 milhões de pessoas são diagnosticadas e tratadas. Estima-se que cerca de 4,8 milhões de pessoas morreram devido às complicações do DM e foram gastos mais de 471 milhões de dólares em cuidados de saúde relacionados com o diabetes.

Em relação à América do Sul e a América Central, o Brasil possui cerca de 12,4 milhões de pessoas com a doença, seguido por Colômbia, Venezuela e Argentina. Em relação aos gastos com a saúde devido ao diabetes nessas regiões, estima-se que foram gastos 20,8 bilhões de dólares e esses valores tendem a aumentar para 32,9 bilhões de dólares em 2030 (FEDERAÇÃO INTERNACIONAL DO DIABETES, 2012).

Estudos mostram que o aumento da prevalência desta doença tem sido relacionado principalmente com mudanças no estilo de vida das pessoas,

aumento do sedentarismo, aumento da obesidade e com o aumento da expectativa de vida da população (SCHMIDT et al., 2009, DIRETRIZES SOCIEDADE BRASILEIRA DE DIABETES (SBD), 2009; SHAW et al., 2010).

De acordo com ZHANG e colaboradores (2010), o DM gera altos custos para o sistema de saúde devido às complicações micro e macrovasculares do DM, tais como: nefropatias, retinopatias, cardiopatias entre outras complicações crônicas e serevas como a demência e transtornos do humor (depressão). Além disso, alguns autores sugerem que são necessários mais esforços para a prevenção do DM, a fim de diminuir os índices de elevação da doença, além de reduzir os gastos relacionados com o DM e suas complicações. Esses altos custos bem como a gravidade do DM apenas ressaltam a necessidade urgente de intervenção e de mais estudos a fim de entender melhor a fisipatologia desta doença.

1.2. CLASSIFICAÇÃO, FISIOPATOLOGIA E DIAGNÓSTICO

De acordo com a Sociedade Brasileira de Diabetes, (2009), a classificação do DM é baseada na etiologia da doença e não mais no tratamento do DM. Assim, sugere-se que os termos insulinoindependente e insulinodependente não sejam mais utilizados. O DM é classificado em três tipos básicos: DM Tipo 1 (DM1), DM Tipo 2 (DM2) e DM Gestacional (MARASCHIN et al., 2010; DIRETRIZES SBD, 2009).

O DM1 representa cerca de 5 a 10 % de todos os casos de DM e é caracterizada por uma deficiência na produção de insulina. Esta deficiência resulta de um processo complexo em que fatores genéticos e ambientais conduzem a uma resposta autoimune levando à destruição das células Beta (β)

pancreáticas produtoras de insulina (BEARDSALL et al., 2006; DAVIES et al., 1994). Em relação ainda ao DM1, há aquele tipo no qual a causa da destruição das células β não é conhecida, sendo denominada como forma idiopática do DM1 (GROOP et al., 1986; IMAGAWA et al., 2000). Geralmente o DM1 inicia antes dos 30 anos de idade, mas pode acometer indivíduos de qualquer faixa etária (BARAT et al., 2008;. VEHIK et al., 2007).

A maior incidência do diabetes é o do DM2 que representa cerca de 90% dos casos, sendo caracterizado pela deficiência na secreção de insulina e/ou resistência à ação da insulina. Esta resistência à ação da insulina é uma condição patológica caracterizada por uma deficiência no mecanismo de sinalização da insulina para a regulação do açúcar no sangue. Diante disso, os tecidos perdem a sensibilidade à ação da insulina e, consequentemente, a concentração de glicose sanguínea aumenta (CAMPBELL et al., 2011; KARAGIANNIS et al.,2012; BEARDSALL et al., 2006; ROSAK, 2002). Esta forma do DM, que acomete pacientes com idade igual ou superior a 40 anos, está relacionada com excesso de peso associado a uma redução na prática de exercícios físicos. Atualmente, tem sido cada vez mais frequente o surgimento de DM2 em crianças e adolescentes devido ao estilo de vida sedentário (WILSON, 2013; RAMKUMAR e TONDON, 2013). Cabe ainda ressaltar que o tratamento com insulina não é essencial, mas pode ser necessário para um melhor controle metabólico, uma vez que a perda da função das células β ocorre progressivamente (DIRETRIZES SBD, 2009; SKYLER et al., 2009).

Outro tipo básico de DM é denominado DM gestacional, o qual afeta as gestantes e é caracterizado por uma diminuição da tolerância à glicose, ou seja, uma deficiência na ação da insulina. Esta diminuição da tolerância à

glicose pode ou não persistir após o parto. Similar ao DM2, o DM gestacional é associado tanto a resistência à insulina quanto à diminuição da função das células β. Interessante mencionar que o DM gestacional ocorre entre 1 a 14% de todas as gestações (LAWRENCE et al., 2008) e está associado com o aumento de morbidade e mortalidade perinatal (DIRETRIZES SBD, 2009).

Sabe-se que o DM está associado a muitas complicações micro e macrovasculares, além das comorbidades já supracitadas. A fim de prevení-las, a Associação Americana de Diabetes modificou em 1997 os critérios para diagnóstico do DM. Posteriormente os critérios foram aceitos pela Organização Mundial da Saúde e pela Sociedade Brasileira de Diabetes. Atualmente são três os critérios aceitos para o diagnóstico de DM:

1. Sintomas de poliúria (aumento do volume urinário), polidipsia (aumento de sede) e perda ponderal de peso, acrescidos de glicemia casual acima de 200 mg/dL. Compreende-se por glicemia casual aquela realizada a qualquer hora do dia, independentemente do horário das refeições;

2. Glicemia de jejum = 126 mg/dL. Em caso de pequenas elevações da glicemia, o diagnóstico deve ser confirmado pela repetição do teste em outro dia;

3. Glicemia 2 h após sobrecarga oral de 75 g de glicose acima de 200 mg/dL. (INZUCCHI, 2012; DIRETRIZES SBD, 2009).

Outro parâmetro importante a ser avaliado é a hemoglobina glicada (HbA1) utilizada como parâmetro de referência para avaliar o grau de hiperglicemia crônica entre os pacientes diabéticos (SACKS, 2003; SAUDEK et al., 2006; CAVAGNOLLI, 2011).

A HbA1 refere-se a um conjunto de substâncias formada pelas reações entre a hemoglobina A (HbA) e alguns açúcares. Este processo de glicação de proteínas envolve uma ligação não enzimática e permanente de açúcares redutores com a glicose (BRY et al., 2001).

A HbA é a forma principal da hemoglobina, sendo que a HbAO é o principal componente da HbA. Por outro lado, HbA1 total corresponde as formas de HbA carregadas mais negativamente devido a adição de glicose ou outros carboidratos. A HbA1 é dividida em três subespécies cromatologicamente distintas: HbA1a, HbA1b e HbA1c, sendo chamadas de maneira geral de HbA1 (NATHAN, 2008; KILPATRICK et al., 2008; BRY et al., 2001).

Segundo alguns autores, há uma estreita correlação entre os níveis de HbA1 e os valores médios da glicose plasmática. Uma elevação de 1% na HbA1 corresponde a, aproximadamente, um aumento médio de 25 a 35 mg/dL na glicemia. As dosagens de glicose e HbA1 são complementares para avaliação do controle do DM, pois fornecem informações distintas e complementares acerca dos níveis de glicemia sanguínea. Enquanto que os resultados de HbA1 refletem a glicemia média no intervalo de dois a três meses antecedentes a coleta, os resultados de glicemia refletem a avaliação pontual, ou seja, no momento da coleta da amostra de sangue (SACKS, 2003; SAUDEK et al., 2006).

Os valores de HbA1 entre 4% e 6% são considerados normais e níveis acima de 7% estão associados a um risco maior de complicações crônicas. (CAVAGNOLLI, 2011). Por esta propriedade, a determinação da HbA1 para

avaliação da glicemia nos diabéticos é de grande utilidade porque avalia o real quadro glicêmico dos últimos meses.

Como já mencionado, muitas das complicações do diabetes podem ser de origem microvascular (devido a danos aos pequenos vasos sanguíneos) e macrovascular (devido a danos em vasos de grande calibre). As principais complicações microvasculares incluem a nefropatia, a retinopatia e a neuropatia. Já as principais complicações macrovasculares incluem doenças cardiovasculares, acidente encefálico vascular e doença vascular periférica (MELENDEZ-RAMIREZ et al., 2010). Outras complicações crônicas do diabetes são a demência (CUKIERMAN et al., 2005) e a depressão (ANDERSON et al., 2001; LEHMANN et al., 1995), sendo este transtorno de humor associado ao diabetes o principal foco de interesse do nosso trabalho.

1.3. COMPLICAÇÕES DO DIABETES: DEPRESSÃO ASSOCIADA AO DM

Estudos clínicos apontam que há uma alta incidência de depressão entre pacientes diabéticos (ANDERSON et al., 2001; CLAVIJO et. al., 2006; NICOLAU et al., 2013). A Depressão, segundo o Manual de Diagnóstico e Estatística das Doenças Mentais, na sua quinta edição feita pela Associação de Psiquiatria Americana (DSM-V, 2013), é diagnosticada pelo número de sintomas presentes, dentre estes: humor deprimido, acentuada diminuição do interesse ou prazer, perda ou ganho de peso corporal, insônia ou hipersonia, agitação ou retardo psicomotor, fadiga ou perda de energia, sentimento de inutilidade ou culpa excessiva, redução da capacidade de pensar ou concentrar, pensamentos recorrentes de morte, ideia suicida ou tentativa de suicídio. Para que a depressão seja diagnosticada é necessário que no mínimo

cinco destes sintomas estejam presentes durante um período de duas semanas, sendo pelo menos um deles o humor deprimido ou a perda do interesse ou prazer.

Pesquisas mostram que há uma alta incidência de transtorno de humor entre pacientes diabéticos com DM1 e com DM2 (DE GROOT et al., 2001; ANDERSON et al., 2002; VON KORFF et al., 2005; NOUWEN et al., 2010; SMITH et al., 2013). O risco de depressão em pacientes com DM é de 15 a 20% maior do que o risco para a população em geral (LUSTMAN et al., 1992; GAVARD et al., 1993). A prevalência de depressão em pacientes com DM2 varia cerca de 11 a 32% (Ali et al., 2006), enquanto em pacientes com DM1 varia de 8 a 12% (Lustman et al., 2005). Na verdade, estas porcentagens devem ser maiores, uma vez que a depressão muitas vezes não é diagnosticada nem tratada em pacientes diabéticos (LUSTMAN et al., 1992, 2005), agravando ainda mais o estado diabético.

Além de evidências clínicas, os estudos pré-clínicos mostram um aumento no comportamento do tipo depressivo em animais com diabetes induzido quimicamente pela injeção de estreptozotocina (STZ) (Gomez e Barros, 2000; Wayhs et al., 2010; Caletti et al., 2012; Ho et al., 2012). Neste sentido, cabe ressaltar que a STZ é um antibiótico de natureza glicosamina-nitrosuréia com propriedades tóxicas e que foi inicialmente isolada e caracterizada como um antimicrobiano de largo espectro a partir de colônias de *Streptomyces achromogenes (*XIANG et al., 2010; SZKUDELSKI, 2001; DELFINO et al., 2002). A injeção dessa substância, a STZ, é capaz de induzir o diabetes, sendo o modelo experimental de indução do DM1 mais utilizado.

A indução deste estado diabético experimental é devido a sua capacidade de destruição das células β pancreáticas levando a diminuição da secreção de insulina (SZKUDELSKI, 2001). A forma pela qual a STZ danifica as células produtoras de insulina se deve a similaridade da molécula de STZ com a molécula de glicose o que permite que a mesma seja internalizada via transportadores de glicose do tipo GLUT2, expressos na superfície das células β pancreáticas (SZKUDELSKI, 2001; KARUNANAYAKE et al., 1976). A ação tóxica intracelular da STZ se dá pelo aumento dos níveis de espécies reativas de oxigênio molecular ocasionando alquilações de bases nitrogenadas que compõem o ácido desoxirribonucleico (DNA) celular (LEDOUX et al., 1986; ELSNER et al., 2000; SZKUDELSKI, 2001; LENZEN, 2008), as quais, quando reparadas, causam alterações no metabolismo de células β por acarretarem diminuição celular de nicotinamida adenina dinucleotídeo (NAD) e consequentemente de adenosina trifosfato (ATP). Assim, este esgotamento da energia celular resulta, em última análise, em necrose das células β pancreáticas (SANDLER e SWENNE, 1983; BÓLZAN e BIANCHI, 2002; DELFINO et al., 2002).

Interessante que o uso da administração da STZ como modelo de diabetes induzido experimentalmente se justifica pela alta capacidade de mimetizar quadros patológicos observados em pacientes diabéticos como poliúria, polifagia e hiperglicemia (GOYARY e SHARMA, 2010).

De interesse para o nosso estudo, evidências mostram que ratos com o diabetes induzido por STZ apresentam um comportamento do tipo depressivo mais expressivo, ou seja, um tempo de imobilidade mais pronunciado quando avaliados no teste de natação forçada (TNF) e comparados com animais

normoglicêmicos (GOMEZ e BARROS, 2000; WAYHS et al., 2010; HAIDER et al., 2013). O mesmo comportamento do tipo depressivo mais pronunciado (aumento de imobilidade) ocorre em camundongos também diabéticos submetidos ao TNF (HILAKIVI-CLARKE et al., 1990; ANJANEYULU et al., 2003) e a outro teste que também avalia o tempo de imobilidade, o teste de suspensão pela cauda (HO et al., 2012). Esse aumento do tempo de imobilidade em ambos os testes comportamentais, indica um comportamento do tipo depressivo que é revertido por drogas antidepressivas. (CRYAN et al., 2002; BLANCHARD et al., 2013). Os mecanismos da relação diabetes/depressão ainda não foram completamente elucidados.

Os sintomas de depressão mais comuns relacionados ao diabetes são: perda de peso, retardo psicomotor, cansaço, sonolência, sentimento de inutilidade e diminuição do apetite sexual (LUSTMAN et al., 1992). É proposto que a depressão em pacientes diabéticos, possa ser resultante de alterações no estilo de vida (restrição dietética, tratamento crônico, aumento em gastos financeiros, aumento em frequência de hospitalização) ou possa estar relacionada às alterações fisiológicas decorrentes do diabetes como cegueira, impotência e perda cognitiva (WREDLING et al., 1992; LOWE et al., 1994).

Estudos apontam que transtornos do humor, como a depressão, são um fator de risco crucial para induzir uma piora no controle da glicemia, uma vez que a depressão está associada com a hiperatividade do eixo Hipotálamo-Pituitária-Adrenal (HPA), elevando os níveis de cortisol em humanos e corticosterona em ratos (CAMERON et al., 1984; BELLUSH et al., 1991; CHAMPANERI et al., 2010). Nesse sentido, uma das primeiras alterações endócrinas identificadas após a administração de STZ é um aumento dos

níveis de corticosterona no plasma de animais diabéticos (DE NICOLA et al., 1976). Em outro estudo, ratos tratados com STZ exibiram aumentos significativos na secreção de corticosterona na urina (SCRIBNER et al., 1993).

Como o cortisol é um hormônio com ação contra-regulatória, a exposição prolongada a esse hormônio induz adiposidade visceral, resistência à insulina, dislipidemia entre outros precursores metabólicos relacionados também com o diabetes (NEMEROFF et al., 1996; BELLUSH et al., 1991; JURUENA et al., 2003; CHAMPANERI et al., 2010). Além disso, esses fatores ainda podem contribuir para o desenvolvimento de complicações diabéticas subsequentes (DE GROOT et al., 2001).

Evidências apontam ainda que a hiperglicemia persistente parece ter um papel importante na fisipatologia de doenças afetivas associados ao DM (WRIGHTEN et al, 2008; BAHRMANN et al., 2012). Tem sido sugerido que a hiperglicemia provoca danos nos tecidos por meio de vários mecanismos, tais como o aumento do fluxo de glicose e outros açúcares, através da via do poliol, o aumento da formação intracelular de produtos finais de glicação avançada (AGEs), o aumento da expressão do receptor para produtos finais de glicação avançada e seus ligantes ativadores, ativação de isoformas da proteína quinase C (PKC) e hiperatividade da via hexosamina (revisado por GIACCO E BROWNLEE, 2010). Diversas linhas de evidências indicam que todos estes mecanismos culminam no aumento do estresse oxidativo (NAUDI et al., 2012; DE CARVALHO et al., 2012).

1.4. ESTRESSE OXIDATIVO

As espécies reativas de oxigênio e nitrogênio (EROs e ERNs respectivamente) são produzidas durante o metabolismo basal das células sendo exemplos dessas espécies o ânion superóxido, o radical hidroxila e o peróxido de hidrogênio. Sob condições normais, nosso organismo possui enzimas protetoras ou antioxidantes que reparam 99% dos danos causados pelas EROs e/ou ERNs (HALLIWELL, 2001).

A definição de antioxidantes é útil para o melhor entendimento dos processos oxidativos. Os antioxidantes podem ser definidos como qualquer substância que irá, dependendo de sua concentração no meio, protelar ou impedir a oxidação de um substrato agindo na prevenção, interceptação e/ou no reparo contra a formação de substâncias nocivas às células ou tecidos (ROHENKOHL et al., 2011). Esses agentes antioxidantes podem ser classificados em enzimáticos, dentre os quais se destacam a enzima superóxido dismutase (SOD) e catalase (CAT), e não enzimáticos como a glutationa reduzida (GSH) e as vitaminas E e C (VALKO et al., 2007)

A SOD é uma enzima antioxidante que catalisa a dismutação do ânion superóxido a peróxido de hidrogênio envolvendo processo sucessivo de oxidação e redução, tornando o produto menos reativo que o anterior, sendo fundamental para a prevenção da toxicidade induzida pelas EROs/ERNs (FRIDOVICH, 1997; STOCKER e KEANEY, 2004). Essa enzima é abundante no organismo e encontra-se no citoplasma das células, utilizando como cofator o cobre e o zinco, estando presente também na mitocôndria sendo o manganês o cofator utilizado nessa organela (FRIDOVICH, 1997). Já a CAT, encontrada

presente nos peroxissomos, apresenta a função de promover a decomposição do produto da ação da SOD, o composto tóxico e reativo peróxido de hidrogênio. Essa enzima catalisa a reação entre duas moléculas de peróxido de hidrogênio, resultando na formação de água e oxigênio molecular (FRIDOVICH, 1997; HALLIWELL e GUTTERIDGE, 2007).

Em relação aos antioxidantes não enzimáticos, como por exemplo, a glutationa (L-γ-glutamil-L-cistenilglicina), a qual é sintetizada principalmente pelo fígado e está presente no organismo em duas formas: reduzida (GSH) e a forma oxidada (GSSG). A GSH é um tiol não proteico constituído de glutamato, cisteína e glicina que participa direta ou indiretamente de diversos processos celulares, tais como a síntese de DNA, proteínas e também da modulação da função proteica (STOCKER e KEANEY, 2004; DRINGEN et al., 2005). A GSH pode servir de substrato para a ação da enzima glutationa peroxidase (GPx) agindo na detoxificação de peróxidos orgânicos e de hidrogênio. Por servir como cofator para diversas enzimas a GSH representa um dos principais compostos endógenos que combatem as EROs (FRIDOVICH, 1997; STOCKER e KEANEY, 2004; MAHER, 2005).

O estresse oxidativo é um desequilíbrio entre a geração de ERO, de ERN e antioxidantes. Este desequilíbrio pode interferir na função de inúmeras macromoléculas, incluindo aquelas que constituem o sistema de transporte de elétron e consequentemente interrompendo a função mitocondrial (MADRIGAL et al., 2006). Além disso, induz um aumento da concentração intracelular de moléculas altamente reativas que provocam danos à estrutura das células por promoverem danos a estrutura proteica, quebra no DNA e também danos a

membrana celular, como a peroxidação lipídica (LPO) (SIES, 1997; VALKO et al., 2007).

Neste último aspecto, a LPO pode ser definida como uma cascata de eventos bioquímicos que provoca a oxidação de lipídios polinsaturados presentes nas membranas celulares (HALLIWELL e GUTTERIDGE, 1999; DAL-PIZZOL et al., 2000). As organelas (como exemplos, mitocôndrias, lisossomos e peroxissomos) e membranas celulares contém grande quantidade de lipídios polinsaturados, o que os tornam componentes celulares facilmente atingíveis pelas EROs e ERNs (HALLIWELL e GUTTERIDGE, 1999; URSO e CLARKSON, 2003). As principais consequências da LPO são as alterações na estrutura e na permeabilidade da membrana. Essas alterações podem levar à destruição da estrutura celular e alteração dos mecanismos de troca de metabólitos. Mais ainda, promover liberação do conteúdo das organelas e formação de produtos citotóxicos o que numa condição extrema pode levar à morte celular (HALLIWELL e GUTTERIDGE, 1999; DAL-PIZZOL et al., 2000).

Cabe ainda citar que o alfa-tocoferol, um dos principais componentes ativos da Vitamina E, é considerado o principal antioxidante lipossolúvel do organismo, cujo principal mecanismo de ação é impedir a peroxidação lipídica, atuando na manutenção da integridade das membranas biológicas, reagindo com radicais peroxil, produtos primários da oxidação de ácidos graxos, impedindo assim a propagação da lipoperoxidação. Assim, o alfa-tocoferol atua protegendo ácidos graxos polinsaturados que estão presentes nos fosfolipídios de membrana e nas lipoproteínas do plasma (MUNTEANU et al., 2004; HONG et al., 2004; TRABER e ATKINSON, 2007).

Estudos indicam que a ação central das ERO/ERN levando à destruição oxidativa dos neurônios está associada a transtornos psiquiátricos (MAES et al., 2000; TSUBOI et al., 2006; BECKER, 2007; VALKO et al., 2007, BEHR et al., 2012). Sabe-se que o encéfalo é particularmente propenso aos danos ocasionados por essas espécies reativas, uma vez que o encéfalo é um tecido altamente rico em ácidos graxos polinsaturados oxidáveis e pobres em defesas antioxidantes. Além disso, este órgão requer alto consumo de oxigênio e há abundância de metais redox-ativos, acompanhado por um déficit relativo em sistemas antioxidantes (VALKO et al., 2007; MANGIALASCHE et al, 2009;.WANG e MICHAELIS, 2010).

Interessante notar que áreas que fazem parte do sistema límbico e que estão envolvidas na fisiopatologia da depressão, como o hipocampo (HIP) e córtex pré-frontal (CPF) são afetadas por esse desequilíbrio (BREMNER et al., 2002; FRODL et al., 2002; MAYBERG et al., 2009; SAVITZ et al., 2009; INNOS et al., 2013). Assim, evidências demonstram que tal como ocorre no diabetes, o desequilíbrio entre EROs/ERNs e enzimas antioxidantes também parece desempenhar um papel importante na patogênese da depressão (IRIE et al., 2003; ATES et al., 2007; LUCCA et al, 2009).

1.5. HIPÓTESE

A depressão associada ao diabetes é uma consequência direta da hiperglicemia e de alterações bioquímicas, como estresse oxidativo no HIP e CPF.

2.0.OBJETIVO

O objetivo do nosso estudo foi investigar o efeito do tratamento prolongado com insulina, vitamina E (antioxidante potente) ou imipramina (antidepressivo tricíclico) sobre respostas comportamentais relacionadas com a depressão e sobre parâmetros do estresse oxidativo avaliados no córtex pré-frontal e hipocampo de animais normoglicêmicos e diabéticos.

3.0. ARTIGO CIENTÍFICO

Increased oxidative stress in hippocampus and prefrontal cortex is related to depressive-like behavior in streptozotocin-diabetic rats.

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3.1 ABSTRACT

Depression is a common comorbid in diabetic patients. The pathophysiologic mechanisms that relate this comorbidity is not completely elucidated yet, although evidences point out that increased oxidative stress resulting from hyperglycemia may have a crucial role. Thus, the effect of prolonged treatment with insulin (INS), the antioxidant vitamin E (VIT E) or the antidepressant imipramine (IMI) was evaluated on behavioral response related to depression and on oxidative stress parameters (lipid peroxidation product levels, reduced gluthatione levels and catalase and superoxide dismutase activities) in prefrontal cortex (PFC) and hippocampus (HIP). Our data show that treatment of streptozotocin-induced diabetic (DBT) rats with INS (6 UI/day, s.c.) prevented the blood glucose increase, reduced the immobility time, an antidepressant-like behavior, and normalized the reduced weight gain. Although the VIT E treatment (300 mg/kg, p.o.) had not altered the blood glucose levels, this treatment was able to reduce the immobility time and to reestablish the reduced weight gain in DBT rats. Differently, treatment with IMI (15 mg/kg, i.p.) induced antidepressant-like behavior in normoglycemic besides DBT animals. While VIT E and IMI treatments restored only specific oxidative stress parameters, INS was able to prevent all changed parameters evaluated in both PFC and HIP from DBT animals. Therefore, our data provide further evidence of the importance of oxidative stress in PFC and HIP in the pathophysiology of depression related to diabetes.

Keywords: diabetes, depression, oxidative stress, vitamin E, insulin, imipramine

3.2 Introduction

Depression is the most common psychiatric disorder identified in patients with diabetes. Besides, the prevalence of major depressive disorder has been recognized to be much higher among people with diabetes compared to non-diabetic populations [1,2,3]. Furthermore, depression, in turn, may increase the risk for hyperglycemia, leading to a poorer management of plasma glucose and an increase in diabetes-related complications, in healthcare expenses and in medical morbidity and mortality [4,5]. Also noteworthy is that depression induces activation of various integrated biological systems that can increase insulin resistance as well as facilitating the development of diabetes [6].

It has recently become clear that the central nervous system (CNS) is not spared from the deleterious effects of diabetes, since diabetic encephalopathy was recognized as a complication of this heterogeneous metabolic disorder [7, for a review, see 8]. In both human and animal models, diabetes is associated with pathological changes in the CNS that lead to cognitive and affective deficits, and to an increased risk of brain vascular complications [9].

Given the high costs of treatment for depression and diabetes, studies have been extensively growing in an attempt to meet an effective treatment to avoid the symptoms and damage caused by both diabetes and depression. In this regard, it is known that maintenance of glucose homeostasis prevents the harmful effects of the disease on the CNS, heart, eyes, kidneys, nerves and peripheral vasculature [10,11]. As rigid glycemic control is not always clinically possible, the antidepressants have been recommended as the first-choice drugs for treating depression associated with diabetes [12]. However, evidences show the treatment with antidepressant drugs is effective in only a subset of

patients with depression (only 30% respond to treatment effectively), and it requires a continuous therapy (weeks to months) to achieve a therapeutic response [13,14]. Moreover, antidepressant drugs can also directly affect plasma glucose and insulin levels besides interact with hypoglycemic drugs [15]. Thus, the understanding of the pathophysiological mechanisms responsible for the development of diabetes and depression is urgent and it may help to propose alternative treatments which aim an increase of effectiveness and reduction of latency to the therapeutic effect.

The persistent hyperglycemia appears to have a major role in the onset of cognitive and affective disorders associated with diabetes [16]. It has been suggested that hyperglycemia causes tissue damage through several mechanisms, such as increase in glucose flux and other sugars through the polyol pathway, increase of advanced glycation end-products (AGEs) synthesis, increase of AGEs receptor expression, activation of protein kinase C isoforms and overactivity of the hexosamine pathway (reviewed by [17]). Furthermore evidences indicate that these mechanisms are activated by increased oxidative stress [18,19], which results from increasing of reactive species of oxygen or nitrogen (ROS/RNS) production and/or impairment of antioxidant defenses [20]. In fact, the oxidative stress can lead to damage of the main components of the cellular structure, including nucleic acids, proteins, amino acids, and lipids [21], affecting several cell functions, such as metabolism and gene expression, which in turn can precipitate or impair other pathological conditions [22]. The persistence of oxidative stress also leads to a cascade of events resulting in neurodegenerative apoptotic injury [23].

Interestingly clinical studies indicate that increased oxidative stress may also contribute to depressive states [24,25,26]. Therefore, antioxidants such as vitamin E and C were observed to be reduced in serum of the depressed patients [27,28]. Corroborating these data, studies show that antioxidants treatment can induce antidepressant effects [29,30,31].

Considering that the association between diabetes and depression may be a direct consequence of hyperglycemia and biochemical changes such as oxidative stress, in an attempt to understand the mechanisms related to the favoring of comorbidity diabetes/depression, the aim of our study was to investigate the effect of prolonged treatment with insulin, vitamin E (a potent antioxidant) or antidepressant drug imipramine on behavioral responses related to depression and on oxidative stress parameters evaluated in the prefrontal cortex and hippocampus.

3.3 Material and methods

3.3.1. Animals

Male *Wistar* rats (200-250 g), provided by the Federal University of Paraná colony, were used. The animals were housed in plastic cages (41 x 32 x 16.5 cm) with four rats per cage and food and water available *ad libitum*. They were maintained in a temperature-controlled room (22 ± 2°C) under 12h/12 h light/dark cycle (lights on at 7 am). The experiments were carried out according to Brazilian Society of Neuroscience and Behavior guidelines for care and use of Laboratory animals and all efforts were made to minimize animal suffering.

The experimental protocol was approved by the local Ethical Committee (CEUA/BIO-UFPR; #576).

3.3.2. Drugs

The following drugs were used: human NPH Insulin (INS; Humulin®, Lilly, USA), Streptozotocin (STZ; Santa Cruz Biotechnology Inc., USA), sodium citrate (Merck S.A., Brazil), tricyclic antidepressant drug Imipramine (IMI; Novartis Pharmaceutical Industry, Brazil) and antioxidant drug Vitamin E (VIT E; Pharma Nostra, Brazil). STZ was dissolved in citrate buffer (10 mM, pH 4.5), IMI and INS were dissolved in saline and VIT E was dissolved in corn oil. The doses and treatment schedules were based on previous studies [32,33] and pilot experiments in our laboratory. Although all experiments were carried out by an observer blind to drug treatments, the experimenter could not be blind to normoglycemic and diabetic groups.

3.3.3. Diabetes induction

Experimental diabetes was induced following an overnight fast by a single intraperitoneal (i.p.) injection of STZ at dose of 50 mg/kg freshly dissolved in citrate buffer (10 mM, pH 4.5). Hyperglycemia was confirmed 72 hours after STZ administration by a strip operated reflectance meter in a blood sample obtained by tail prick and confirmed again at ending of the behavioral tests. Only the animals with fasting blood glucose levels ≥ 250 mg/dL were maintained in the study [34]. All animals were observed daily and weighed regularly during the experiment.

3.3.4. Open-field test

Open-field test was conducted according to Santiago and collaborators (2010) [35]. Briefly, animals were placed in the center of a rectangular open field (40 × 50 × 63 cm) with a floor divided into 6 rectangular units. The exploratory activity was recorded during 5 minutes and the number of squares crossed with all four paws was quantified.

3.3.5. Forced swimming test (FST)

Independent groups of rats were submitted to FST as described by Porsolt and collaborators (1978) [36] with minor modifications. The test was conducted in two sessions. First, in the pre-test session rats were placed individually to swim in a tank (30 cm diameter by 40 cm height containing 25 cm of water at 24 ± 1°C) for 15 min. Twenty four hours later, animals were submitted to a 5 min session of forced swim (test). During this session, total time of immobility (except small movements necessary to float) and the latency to the first immobility episode were evaluated [37]. After each session (pre-test and test session), the animals were removed and allowed to dry in a separate cage before being returned to their home cages.

3.3.6. Preparation of subcellular fractions of brain

Prefrontal Cortex (PFC) and hippocampus (HIP) from NGL and DBT animals were dissected and homogenized in 200 mM of potassium phosphate buffer (pH 6.5). The homogenate was used to determine the reduced glutathione (GSH) and lipid hydroperoxides (LOOH) levels and then centrifuged at 9000 × *g*

for 20 min. The supernatant was used for the determination of superoxide dismutase (SOD) activity and catalase (CAT) activity.

3.3.7. Protein assay

Protein concentrations were determined by the Bradford method (Bio-Rad, Hercules, CA, USA), using bovine serum albumin as standard and carried according to the manufacturer's instructions.

3.3.8. Determination of lipid hydroperoxides (LOOH) content

The content of LOOH, a highly reactive product of lipid peroxidation, was determined by the Ferrous Oxidation-Xylenol Orange (FOX2) method as previously described [38]. Briefly, 10 µl of 90% methanol was added to 100 µl of homogenate, sonicated and centrifuged at 9000 × *g* for 20 minutes at 4°C. The supernatant was mixed with FOX2 reagent and incubated for 30 min at room temperature. The absorbance was determined at 560 nm and the results were expressed as mmol/mg of tissue.

3.3.9. Determination of reduced glutathione (GSH) levels

GSH levels in PFC and HIP were determined as previously described [39]. Aliquots of tissue homogenate were mixed with 12.5% trichloroacetic acid, vortexed for 10 min and centrifuged for 15 min at 900 × *g*. Subsequently, the supernatant were mixed with TRIS buffer (0.4 M, pH 8.9) and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, 0.01 M). Absorbance was measured by spectrophotometry at 415 nm with a microplate reader. The procedures were

performed at 4 °C, and the individual values were interpolated into a standard curve of GSH (0.375–3 µg) and expressed as µg/g of tissue.

3.3.10.Determination of superoxide dismutase (SOD) activity

The method used to determine SOD activity is based in the capacity of SOD to inhibit pyrogallol autoxidation, as previously described [40,41]. Then, pyrogallol (1 mM) was added to buffer solution (200 mM Tris HCl–EDTA, pH 8.5) and to supernatant aliquots, and then vortexed for 1 min. The reaction was incubated for 20 min at room temperature, stopped with the addition of 1 N HCl and centrifuged for 4 min at 18700 × g. Absorbance was measured at 405 nm. The amount of SOD that inhibited the oxidation of pyrogallol by 50%, relative to the control, was defined as one unit of SOD activity. The results were expressed as U/mg of protein.

3.3.11. Determination of catalase (CAT) activity

CAT activity in PFC and HIP were determined as previously described [42]. Briefly, 10 µl of supernatant aliquots was added to 990 µl of the reaction buffer containing 1 mM Tris, 5 mM EDTA and 30% H_2O_2 (pH 8.5) and vortexed for 1 min. Then, the decrease in optical density due to decomposition of H_2O_2 was measured at 240 nm and was recorded for calculation of the CAT activity. CAT activity was defined as the amount of enzyme required to decompose 1 nM of H_2O_2 per minute, at 25°C. Results are expressed as millimole per minute per milligram of protein (mmol. min -1 /mg of protein).

3.3.12. Glycated Hemoglobin

Glycated hemoglobin was determined by ion-exchange resin according to the manufacturer instructions (*In vitro* Diagnóstica Ltda, Itabira, MG, Brazil). Briefly, the blood samples were mixed with lysing reagent to prepare a hemolysate. The hemolyzed preparation is then mixed for 5 minutes with a cationic resin which binds hemoglobin (HbAO). After centrifugation, glycated hemoglobin (HbA1) is determined in the supernatant. The absorbance was determined at 405 nm and the result was expressed as percentage of HbA1.

3.3.13. Experimental procedure

Insulin (INS; 6 UI/day, s.c., being 2 UI in the morning and 4 UI in the afternoon) or vitamin E (VIT E; 300 mg/kg, p.o.; once a day) treatments were initiated after confirmation of hyperglycemia (72 h after STZ treatment; DBT rats) or 72 h after citrate buffer treatment (normoglycemic - NGL group) for 28 days. Control rats from NGL and DBT rats received equivalent injection of INS or VIT E vehicle (saline or corn oil, respectively) during 28 days. The pre-test session in the FST was conducted on the 30^{th} day after the treatment with citrate buffer (NGL rats) or STZ (DBT rats). On the following day and two hours (INS or saline) or 40 minutes (VIT E or corn oil) after the last injection, the animals were submitted to the open field test, followed by FST. Thirty minutes after the FST and immediately after the last determination of glycemia, the animals were euthanized by decapitation and the brain rapidly removed and placed on ice. The PFC and HIP were dissected, weighed and kept frozen until the biochemical analysis of SOD and CAT activities, GSH and LOOH levels.

Specifically for animals chronically treated with INS, additional blood samples were collected for glycated hemoglobin measurement.

Another set of experiments was performed treating the animals with the antidepressant Imipramine (IMI), used as a positive control to the antidepressant effect. Both NGL and DBT rats remained untreated for 14 days, and then treated during 14 days with saline (equivalent volume) or IMI (15 mg/kg/ml; i.p.) once a day (between 8 a.m. and 9 a.m.). The pre-test session in the FST was conducted on the 30th day after the treatment with citrate buffer (NGL rats) or STZ (DBT rats). On the following day and one hour after the last injection of IMI or saline the animals were submitted to the open field test, followed by FST. Thirty minutes after the FST, the procedure following euthanasia was similar as described above.

3.3.14. Data analysis

The Kolmogorov-Smirnov and Levene tests were initially employed to ensure that the data satisfied the criteria for carrying out ANOVA. When criteria were satisfied, the results are reported as the mean ± SEM. The data were analyzed by two-way analysis of variance (ANOVA) with treatment and condition (normoglycemic and diabetic) as independent factors. When appropriated, Newman-Keuls tests were used for *post-hoc* analyses. Differences were considered statistically significant when $p < 0.05$.

3.4 Results

3.4.1. Effect of prolonged treatment with insulin in normoglycemic and diabetic animals submitted to FST.

The prolonged treatment with insulin (INS) beyond the condition of animals (DBT and NGL) showed a significant effect in the immobility time of animals evaluated on FST [interaction effect between the factors: $F(1,31) = 4.80$; $p < 0.05$], see Fig. 1 (panel A). Newman-Keuls test showed that DBT animals exhibited an increase of immobility time when compared to NGL animals ($p < 0.05$) which was prevented by INS treatment ($p < 0.05$). Also, two-way ANOVA showed a significant interaction effect between treatment and condition factors when the latency of first immobility was evaluated (Fig. 1; panel B) [$F(1,31) = 4.34$; $p < 0.05$]. Thus, *post-hoc* analysis showed that DBT animals expressed a significant reduction of immobility first latency when compared to NGL animals ($p < 0.05$) which was prevented by INS treatment ($p < 0.05$).

As shown in Fig. 1 (panel C), two-way ANOVA showed interaction effect between treatment and condition factors on weight gain [$F(1,31) = 13.05$; $p < 0.05$]. In fact, Newman Keuls test showed that DBT animals had a decreased weight gain compared to NGL animals ($p < 0.05$). Interesting, the INS treatment prevented the reduced weight gain in DBT animals ($p < 0.05$). Regarding to blood glucose levels (Fig. 1, panel D), a significant difference between condition and treatment factors [$F(1,31) = 313.38$; $p < 0.05$] was obtained. Thus, DBT animals exhibited an increase in blood glucose level when compared to NGL ($p < 0.05$) which was normalized after the prolonged treatment with INS. As

expected, the INS prolonged treatment in NGL animals induced a significant decrease in the blood glucose levels ($p < 0.05$).

In relation to percentage of glycated hemoglobin (HbA1; Fig. 2), two-way ANOVA showed interaction effect between treatment and condition factors [$F(1,31) = 45.09$; $p < 0.05$]. After *post hoc* analysis, we found that DBT animals had a higher percentage of HbA1 compared with NGL animals ($p < 0.05$). Also, prolonged treatment with INS prevented the higher percentage of HbA1 in DBT animals ($p < 0.05$) which did not occur in NGL animals.

Regarding to the number of crossings evaluated in the open field test, in spite of two-way ANOVA shows a significant difference in treatment factor [$F(1,31) = 6.64$; $p < 0.05$], the *post hoc* analysis of Newman Keuls did not show any difference between the groups (Table 1).

Effect of condition (NGL or DBT) and treatment (INS, VIT E, IMI or VEH) on number of crossings evaluated in the open field test.

Condition/Drug Treatment	Number of crossings
NGL/VEH	44.3 ± 4.2
NGL/INS	37.3 ± 3.3
DBT/VEH	41.5 ± 4.5
DBT/INS	39.3 ± 2.9
NGL/VEH	54.8 ± 2.8
NGL/VIT E	57.8 ± 4.0
DBT/VEH	47.1 ± 3.4
DBT/VIT E	48.1 ± 2.3
NGL/VEH	47.5 ± 3.7
NGL/IMI	35.0 ± 2.8 *
DBT/VEH	45.5 ± 3.6
DBT/IMI	30.8 ± 3.4 * #

Table 1: Results are expressed as mean ± SEM; *n*= 6-9
* $p < 0.05$ when compared to NGL/VEH.
# $p<0.05$ when compared to DBT/VEH.

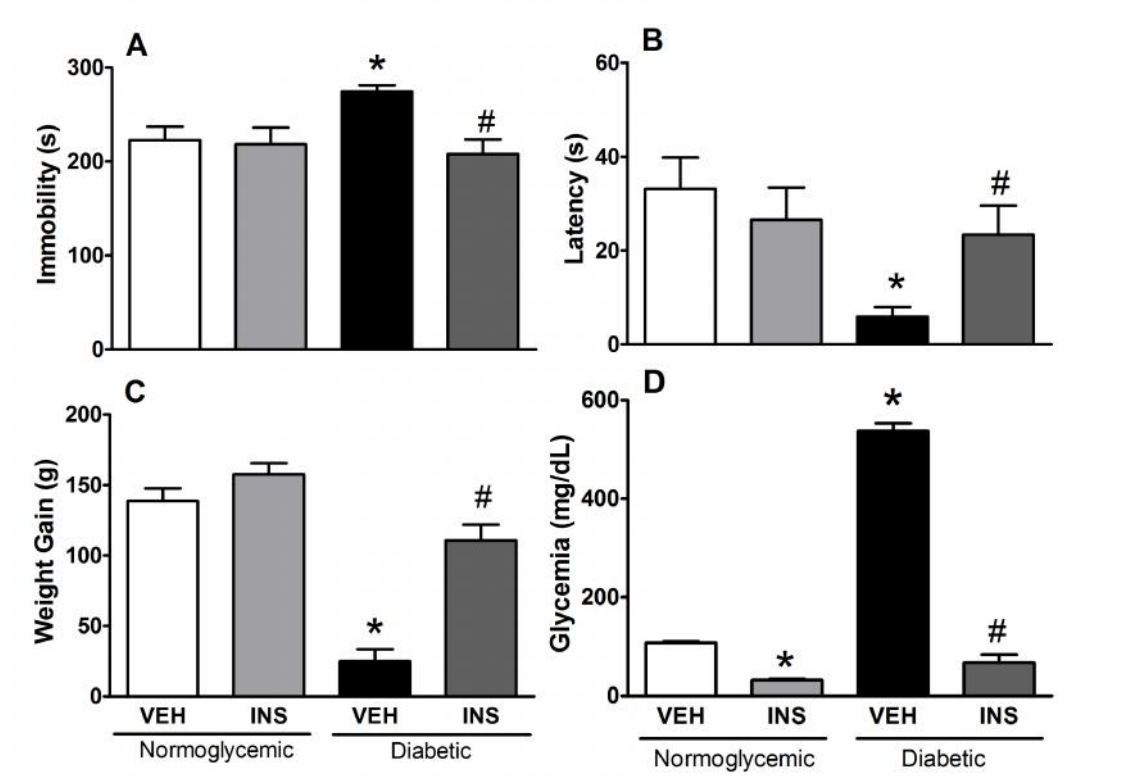

Fig. 1: Effect of prolonged treatment with insulin (INS, 6 IU/day, s.c.) or vehicle (VEH) on the immobility time (s) (A), latency to first immobility (s) (B), weight gain (g) (C) and glycemia (mg/dL) (D) in normoglycemic and diabetic animals. Results are expressed as mean ± SEM, n = 8-9. *$p < 0.05$ compared to the NGL/VEH; $^{\#}p < 0.05$ compared to the DBT/VEH.

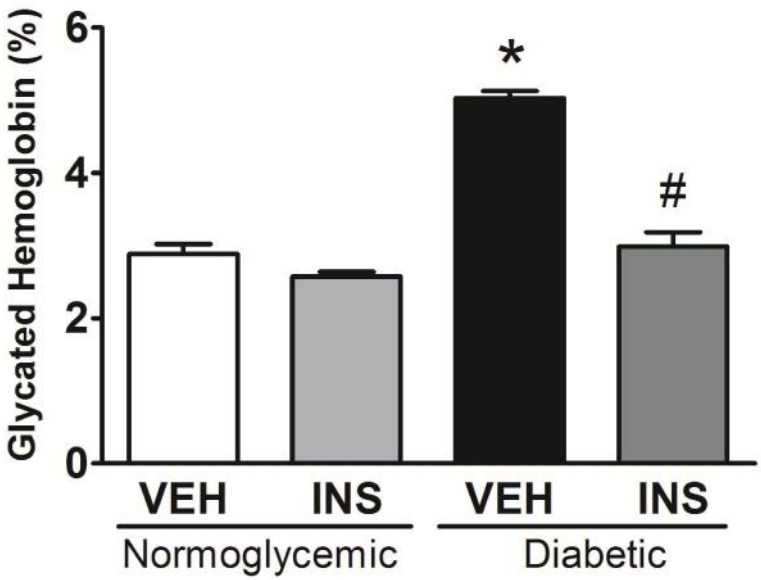

Fig. 2: Effect of prolonged treatment (28 days) with insulin (INS, 6 IU/day, s.c.) or vehicle (VEH) on percentage of glycated hemoglobin normoglycemic and diabetic animals. Results are expressed as mean ± SEM, n = 8-9. *****$p < 0.05$ compared to the NGL/VEH; $^{\#}p < 0.05$ compared to the DBT/VEH.

3.4.2. Effect of prolonged treatment with vitamin E in normoglycemic and diabetic animals submitted to FST.

As shown in Fig. 3 (panel A), the treatment with VIT E significantly altered the immobility time of animals evaluated in the FST [interaction effect between treatment and condition: $F(1,23) = 4.60$; $p < 0.05$]. Newman-Keuls test showed that DBT animals expressed an increased immobility time when compared to NGL animals ($p < 0.05$). Treatment with VIT E prevented this increase in immobility time, an antidepressant-like effect, but only in DBT animals ($p < 0.05$). Corroborating the immobility time data, two-way ANOVA showed significant effect of treatment and condition factors when the latency of first

immobility was evaluated (Fig. 3; panel B) [treatment effect: $F(1,23) = 5.77$; $p < 0.05$; condition effect: $F(1,23) = 9.87$; $p < 0.05$]. Newman-Keuls *post-hoc* test showed that DBT animals had a greater reduction in immobility first latency, when compared with NGL animals ($p < 0.05$). Treatment with Vit E exerted antidepressant-like effect only in DBT animals by increasing the latency of first immobility ($p < 0.05$).

Two-way ANOVA showed that weight gain was altered by different treatment and condition factors [interaction effect: $F(1,23) = 8.02$; $p < 0.05$]. As shown in Fig. 3 (panel C) DBT animals had a reduced weight gain compared to NGL animals ($p < 0.05$) which was prevented by VIT E treatment ($p < 0.05$). Regarding to blood glucose levels, as shown in Fig. 3 (panel D) a significant difference was observed but only in the condition factor [$F(1,23) = 323.81$; $p < 0.05$]. The DBT animals exhibited an increase in blood glucose level when compared to NGL animals ($p < 0.05$) and the treatment with VIT E was not able to prevent this hyperglycemic state.

As shown in Table 1, the number of crossing in the open field was not significantly different among the groups tested (treatment effect: $F(1,23) = 0.02$; $p > 0.05$; condition effect: $F(1,23) = 3.74$; $p > 0.05$).

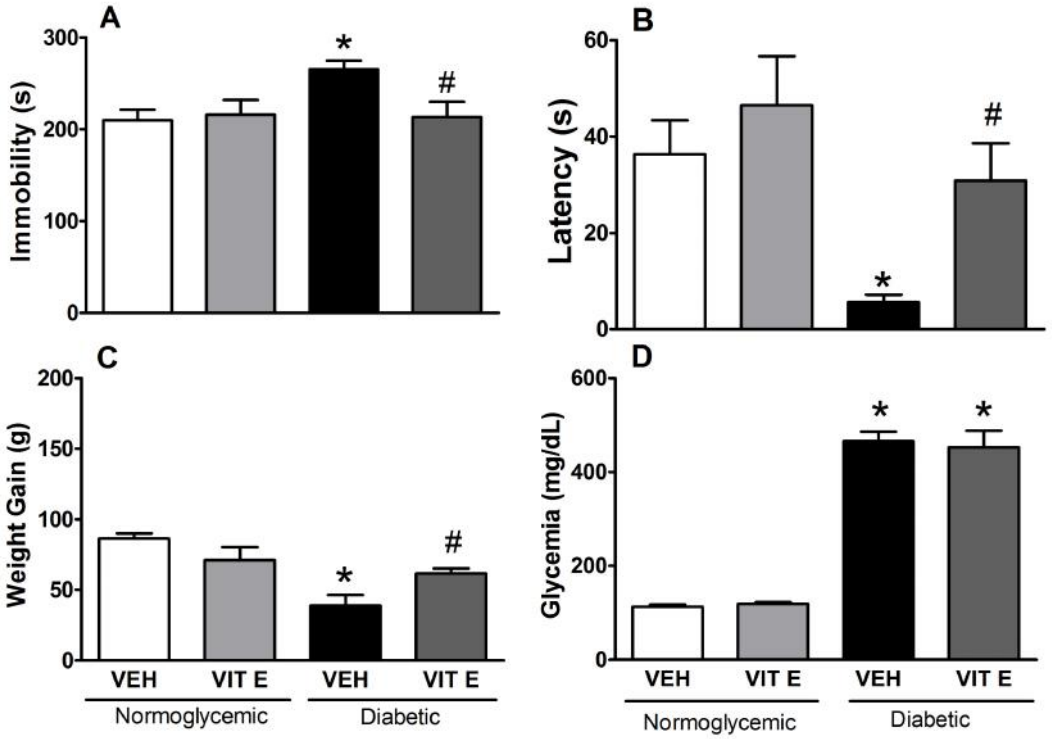

Fig. 3: Effect of prolonged treatment (28 days) with vitamin E (VIT E, 300 mg/kg/day, p.o.) or vehicle (VEH) on the immobility time (s) (A), latency to first immobility (s) (B), weight gain (g) (C) and glycemia (mg/dL) (D) normoglycemic and diabetic animals. Results are expressed as mean ± SEM, n = 6-7. ***p** < 0.05 compared to the NGL/VEH; $^{\#}p < 0.05$ compared to the DBT/VEH.

3.4.3. Effect of prolonged treatment with imipramine in normoglycemic and diabetic animals submitted to FST.

As can be seen in Fig. 4 (panel A), two-way ANOVA showed a significant treatment [F(1,26) = 17.17; $p < 0.05$] and condition [F(1,26) = 16.69; $p < 0.05$] factors effects on immobility time. Newman-Keuls test showed that DBT animals expressed an increased immobility time when compared with NGL animals ($p <$

0.05). Imipramine (IMI) treatment decreased the immobility time in both NGL and DBT groups when compared with their respective controls ($p < 0.05$).

In the same way, Fig. 4 (panel B) shows that IMI treatment changed the latency of first immobility of NGL and DBT animals [treatment effect: $F(1,26) = 36.86$; $p < 0.05$; condition effect: $F(1,26) = 9.29$; $p < 0.05$]. *Post-hoc* analyses showed that DBT rats presented a lower latency of first immobility when compared to NGL ($p < 0.05$). Differently of INS and VIT E treatments, IMI treatment increased the latency of first immobility in both NGL and DBT animals ($p < 0.05$).

As shown in the Fig. 4 (panel C), two-way ANOVA showed a significant difference on weight gain between DBT and NGL animals [condition effect: $F(1,26) = 56.62$; $p < 0.05$]. Newman Keuls test revealed that DBT animals had a reduction in the weight gain compared to NGL animals. Neither DBT nor NGL animals had the weight gain affected by IMI treatment.

Fig. 4 (panel D) shows that glucose levels were altered between NGL and DBT animals [$F(1,26) = 322.99$; $p<0.05$]. Two-way ANOVA followed by Newman Keuls test showed that DBT animals treated with vehicle exhibited a significant increase in the glucose level when compared to NGL treated with vehicle ($p < 0.05$). Moreover, the treatment with IMI did not change the increased glucose level of DBT animals ($p < 0.05$).

Two-way ANOVA showed (Table 1) that treatment factor induced a significant effect on number of crossings in the open field test ($F(1,26) = 19.45$; $p < 0.05$). Thus, Newman-Keuls multiple comparison test showed a decrease in the number of crossings in the open field, in both animals treated with IMI, DBT and NGL animals ($p < 0.05$).

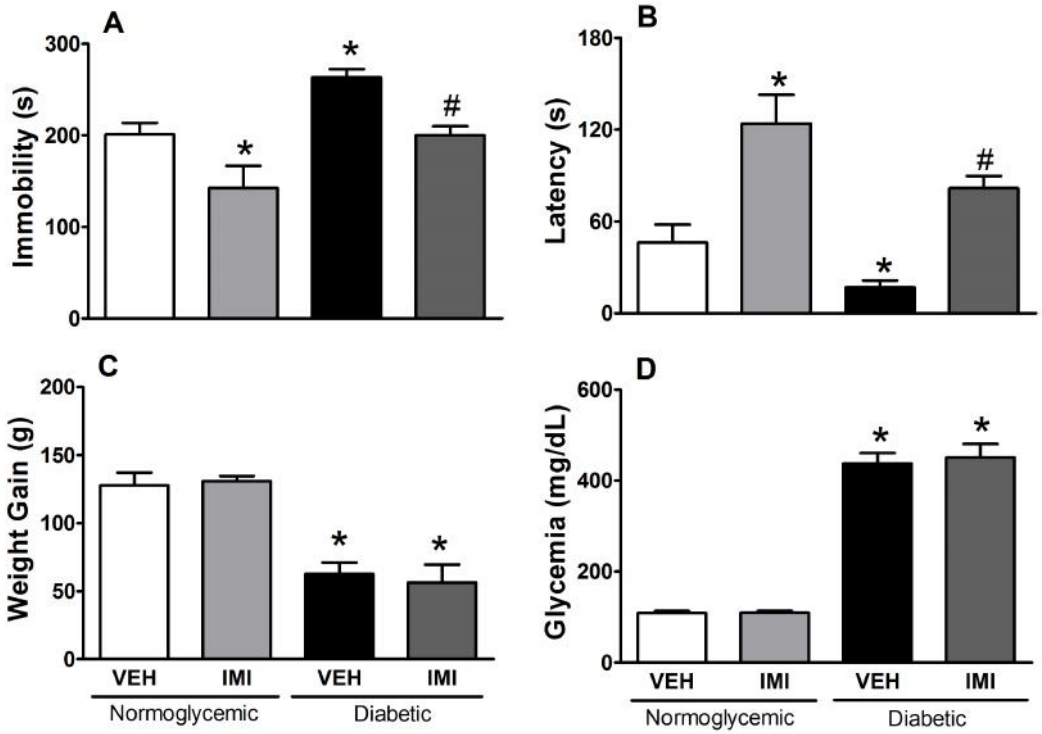

Fig. 4: Effect of treatment with imipramine (IMI, 15 mg/kg, ip, 14 days) or vehicle (VEH) on the immobility time (s) (A), latency to first immobility (s) (B), weight gain (g) (C) and glycemia (mg/dL) (D) in normoglycemic and diabetic animals. Results are expressed as mean ± SEM, n = 7-8. *****$p < 0.05$ compared to the NGL/VEH; $^{\#}p < 0.05$ compared to the DBT/VEH.

3.4.4. Indirect measurements of oxidative stress

3.4.4.1. Effect of prolonged treatment with Insulin, Imipramine or Vitamin E on LOOH contents in prefrontal cortex (PFC) and hippocampus (HIP) from DBT or NGL animals.

The effect of INS treatment on the LOOH content in PFC and HIP is shown in the Fig 5 (panels A and B, respectively). Two-way ANOVA revealed a significant effect of the condition factor on LOOH content in PFC [$F(1,29) = 36.07$; $p < 0.05$] and HIP [$F(1,23) = 32.51$; $p < 0.05$] besides treatment factor

[PFC: $F(1,29) = 42.44$; $p < 0.05$; HIP: $F(1,23) = 8.54$; $p < 0.05$]. Newman Keuls *post-hoc* test revealed that DBT animals exhibited a significant increase in LOOH levels when compared to NGL animals ($p < 0.05$). INS treatment was able to prevent the increased LOOH levels observed in PFC and HIP of DBT animals. Moreover, INS treatment also decreased LOOH content in PFC of NGL animals ($p < 0.05$).

The effect of VIT E treatment on the LOOH content in PFC and HIP is shown in Fig. 5 (panels C and D, respectively). Two-way ANOVA showed a significant effect of the treatment and condition factors in PFC [interaction effect: $F(1,20) = 5.17$; $p < 0.05$] and HIP [interaction effect: $F(1,22) = 7.33$; $p < 0.05$]. Again, Newman Keuls *post-hoc* test showed that DBT animals expressed an increase in LOOH levels when compared with NGL animals ($p < 0.05$), which was prevented by VIT E treatment in both brain areas ($p < 0.05$).

As shown in Fig. 5 (panels E and F), two-way ANOVA revealed a significant interaction effect between treatment and condition factors [PFC: $F(1,26) = 9.78$; $p < 0.05$; HIP: $F(1,25) = 19.51$; $p < 0.05$]. Newman Keuls *post-hoc* test showed that DBT animals had the LOOH levels increased when compared with NGL animals ($p < 0.05$). Also, treatment with IMI significantly reversed the increased LOOH levels observed in PFC and HIP of DBT animals.

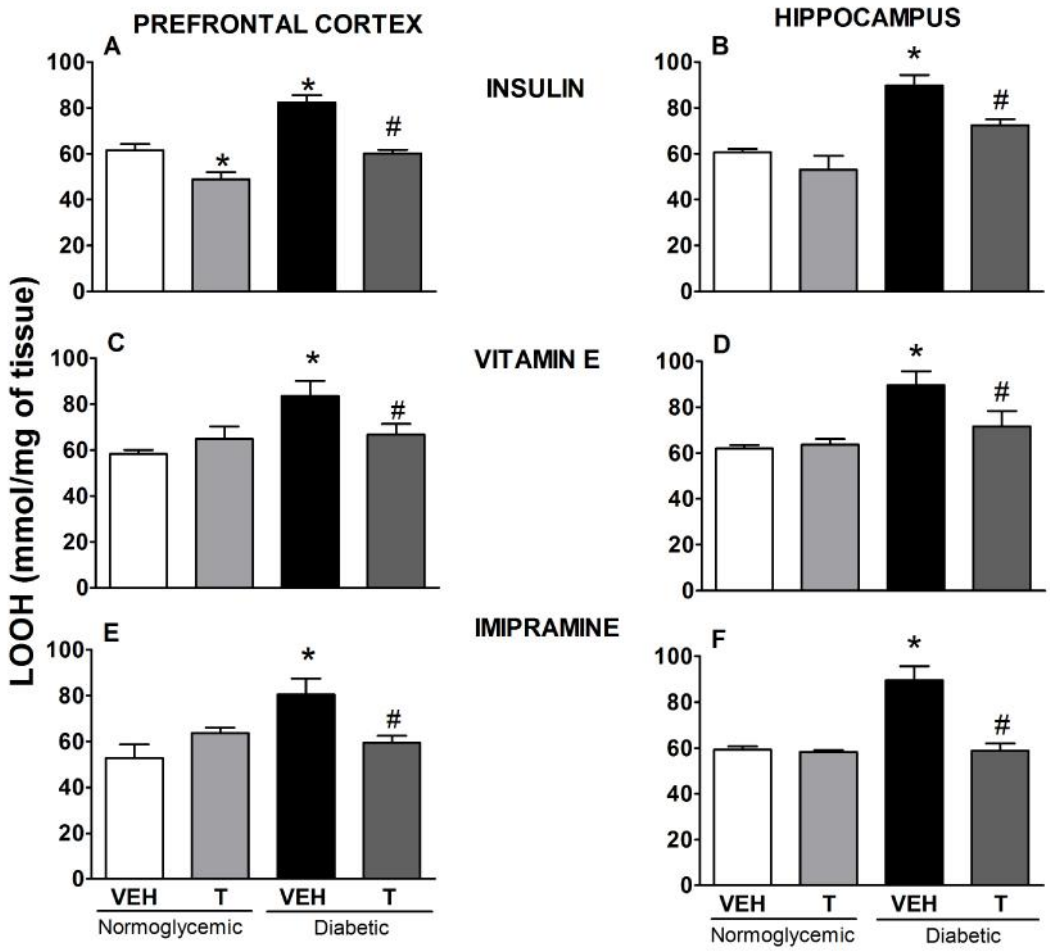

Fig. 5: Effect of treatment (T) with insulin (INS, panels A and B), vitamin E (VIT E, panels C and D), imipramine (IMI, panels E and F) or their respective vehicle (VEH) on the LOOH levels (mmol/mg of tissue) in prefrontal cortex and hippocampus from normoglycemic and diabetic animals. Results are expressed as mean ± SEM, n = 6-9. *****$p < 0.05$ compared to the NGL/VEH; $^{\#}p < 0.05$ compared to the DBT/VEH.

3.4.4.2. Effect of prolonged treatment with Insulin, Vitamin E or Imipramine on the reduced GSH levels in PFC and HIP from DBT or NGL animals

The effect of INS administration on the GSH levels in the PFC and HIP is shown in Fig 6 (panels A and B, respectively). Two-way ANOVA revealed a

significant effect of treatment factor [PFC: $F(3,31) = 21.77$; $p < 0.05$; HIP: $F(3,32) = 5.211$; $p < 0.05$]. Newman Keuls *post-hoc* test showed that DBT animals exhibited a reduction of reduced GSH levels in PFC and HIP, when compared to NGL animals ($p < 0.05$). Moreover, INS treatment prevented the reduced GSH levels in the PFC and HIP of DBT animals ($p < 0.05$) and increased the GSH levels in NGL animals ($p < 0.05$).

The effect of treatment with VIT E on GSH levels in the PFC and HIP is shown in Fig. 6 (panels C and D, respectively). Two-way ANOVA also revealed a significant interaction between treatment and condition factors in PFC [$F(1,24) = 10.77$; $p < 0.05$] and HIP [$F(1,22) = 10.40$; $p < 0.05$]. Newman Keuls test showed that DBT animals expressed reduced GSH levels when compared with NGL animals ($p < 0.05$). Treatment prevented the reduced GSH levels of DBT animals in the PFC as well as in the HIP.

As shown in Fig. 6 (panels E and F), two-way ANOVA revealed a significant effect of IMI treatment in PFC [$F(1,21) = 17.83$; $p < 0.05$] and interaction between treatment and condition factors in HIP [$F(1,23) = 7.51$; $p < 0.05$]. Newman Keuls test showed that the GSH level was reduced in DBT animals when compared with NGL animals ($p < 0.05$). Treatment with IMI restored the GSH levels in the PFC and HIP from DBT animals.

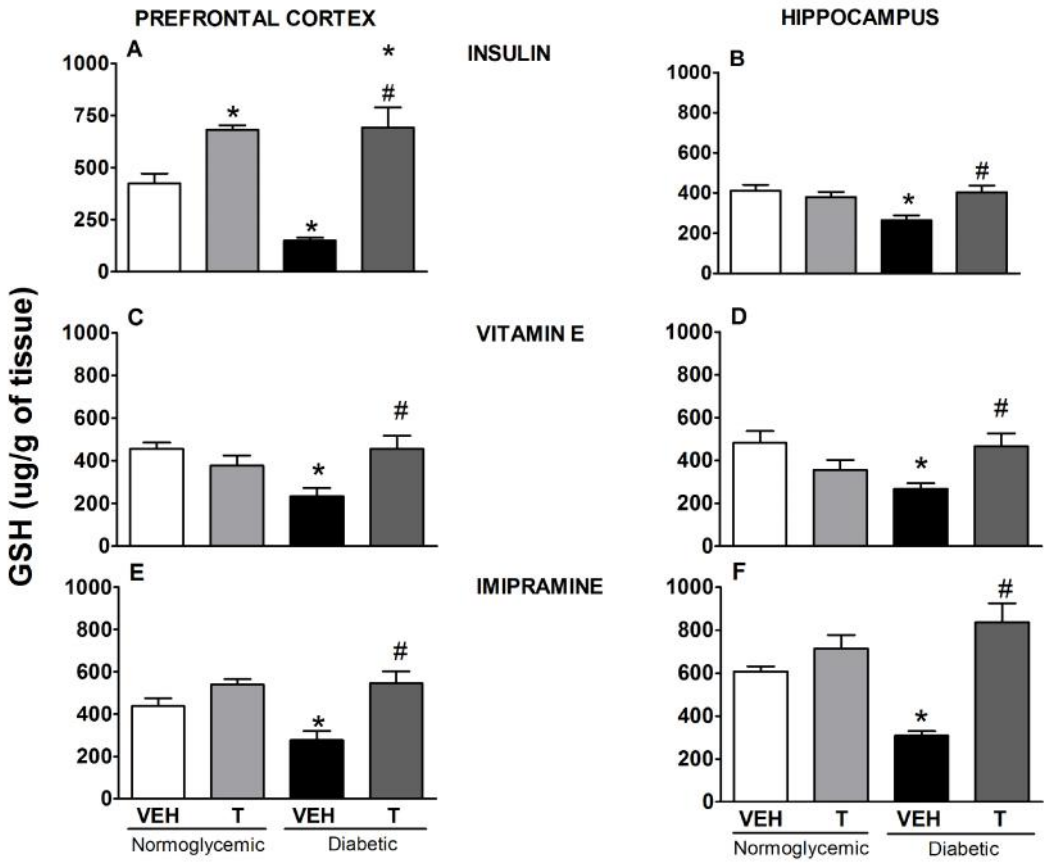

Fig. 6: Effect of treatment (T) with insulin (INS, panels A and B), vitamin E (VIT E, panels C and D), imipramine (IMI, panels E and F) or their respective vehicle (VEH) on the GSH levels (µg/g of tissue) in prefrontal cortex and hippocampus from normoglycemic and diabetic animals. Results are expressed as mean ± SEM, n = 6-9. *****$p < 0.05$ compared to the NGL/VEH; $^{\#}p < 0.05$ compared to the DBT/VEH.

3.4.4.3. Effect of prolonged treatment with Insulin, Vitamin E or Imipramine on the SOD activity in PFC and HIP from DBT or NGL animals

The effect of prolonged treatment with INS on SOD activity in the PFC and HIP is shown in Fig 7 (panels A and B, respectively). Two-way ANOVA revealed a significant interaction effect of treatment and condition factors in PFC [F(1,23)

= 16.00; $p < 0.05$] and HIP [F(1,23) = 28.93; $p < 0.05$]. Newman Keuls *post-hoc* test showed that DBT animals exhibited an increase in SOD activity when compared to NGL animals in both areas, PFC and HIP ($p < 0.05$). Moreover, while INS treatment decreased the SOD activity in the PFC of NGL and DBT animals ($p < 0.05$) the treatment decreased the SOD activity in the HIP, but only of DBT animals ($p < 0.05$).

As shown in Fig. 7 (panels C and D), two-way ANOVA revealed a significant effect only of condition factor [PFC: F(1,25) = 27.06; $p < 0.05$]; HIP: [F(1,20) = 26.02; $p < 0.05$]. Newman Keuls test showed that DBT animals expressed an increase of SOD activity in the PFC and HIP when compared to NGL animals ($p < 0.05$) that was not prevented by VIT E treatment ($p > 0.05$).

As shown in Fig. 7 (panels E and F), two-way ANOVA revealed a significant effect of IMI treatment and condition factors in PFC [treatment effect: F(1,22) = 8.86; $p < 0.05$; condition effect: F(1,22) = 17.26; $p < 0.05$] and interaction between the factors in HIP [F(1,21) = 14.60; $p < 0.05$]. Newman Keuls *post-hoc* test showed that DBT animals expressed an increase of SOD activity when compared to NGL animals ($p < 0.05$). Treatment with IMI restored the SOD activity in the PFC and HIP of DBT animals ($p < 0.05$).

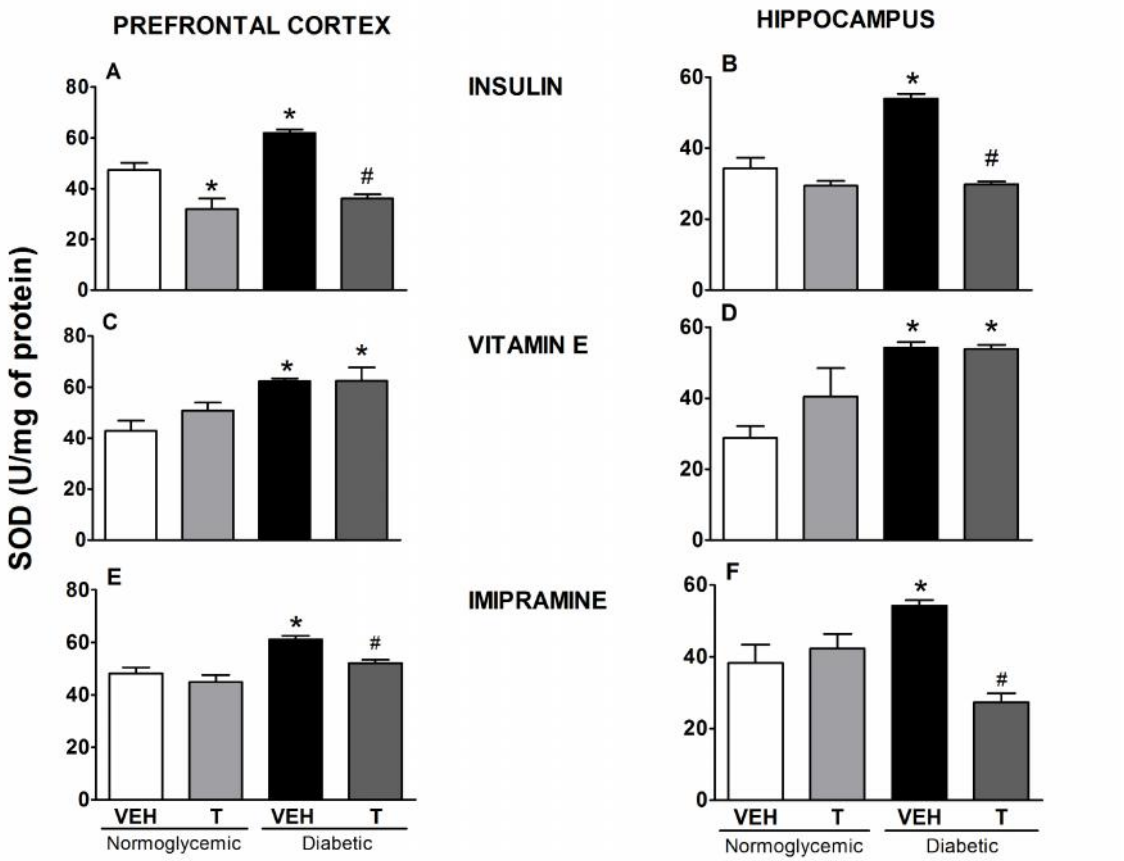

Fig. 7: Effect of treatment (T) with insulin (INS, panels A and B), vitamin E (VIT E, panels C and D), imipramine (IMI, panels E and F) or their respective vehicle (VEH)on the SOD activity (U/mg of protein) in prefrontal cortex and hippocampus from normoglycemic and diabetic animals. Results are expressed as mean ± SEM, n = 6-9. n=6-9. $^{*}p < 0.05$ compared to the NGL/VEH, $^{\#}p < 0.05$ compared to the DBT/VEH.

3.4.4.4 Effect of prolonged treatment with Insulin, Vitamin E or Imipramine on the CAT activity in PFC and HIP from DBT or NGL animals

The effect of prolonged treatment with INS on CAT activity in the PFC and HIP is shown in Fig. 8 (panels A and B, respectively). Two-way ANOVA revealed a significant interaction effect of treatment and condition factors in

PFC [F(1,26) = 10.56; $p < 0.05$] and HIP [F(1,26) = 32.25; $p < 0.05$]. Newman Keuls *post-hoc* test showed that DBT animals exhibited an increase in CAT activity when compared to NGL animals in both areas, PFC and HIP ($p < 0.05$). Moreover, INS treatment prevented the increased CAT activity in the PFC and HIP of DBT animals ($p < 0.05$).

The effect of VIT E treatment on CAT activity in the PFC and HIP is shown in Fig 8 (panels C and D, respectively). Two-way ANOVA also revealed a significant interaction effect between treatment and condition factors [PFC: F(1,23) = 5.16; $p < 0.05$]. Regarding to the HIP, two-way ANOVA showed treatment [F(1,21) = 5.37; $p < 0.05$] and condition [F(1,21) = 16.57; $p < 0.05$] effects but not interaction effect between the factors. Newman Keuls *post-hoc* test showed that DBT animals expressed an increase of CAT activity in the PFC and HIP when compared to NGL animals ($p < 0.05$). VIT E treatment did not prevent the increased CAT activity neither in PFC nor in HIP from DBT animals ($p > 0.05$). Also, VIT E treatment increased the CAT activity in HIP and PFC of NGL animals ($p < 0.05$).

As shown in Fig. 8 (panels E and F), two-way ANOVA revealed a significant interaction effect between treatment and condition factors in PFC [F(1,24) = 8.32; $p < 0.05$] and condition effect in HIP [F(1,24) = 26.68; $p < 0.05$]. Newman Keuls *post-hoc* test showed that DBT animals expressed an increase of CAT activity when compared to NGL animals ($p < 0.05$). Treatment with IMI did not restore the CAT activity in the PFC and HIP of DBT animals ($p > 0.05$). Moreover, in NGL animals IMI treatment was able to increase the CAT activity ($p < 0.05$).

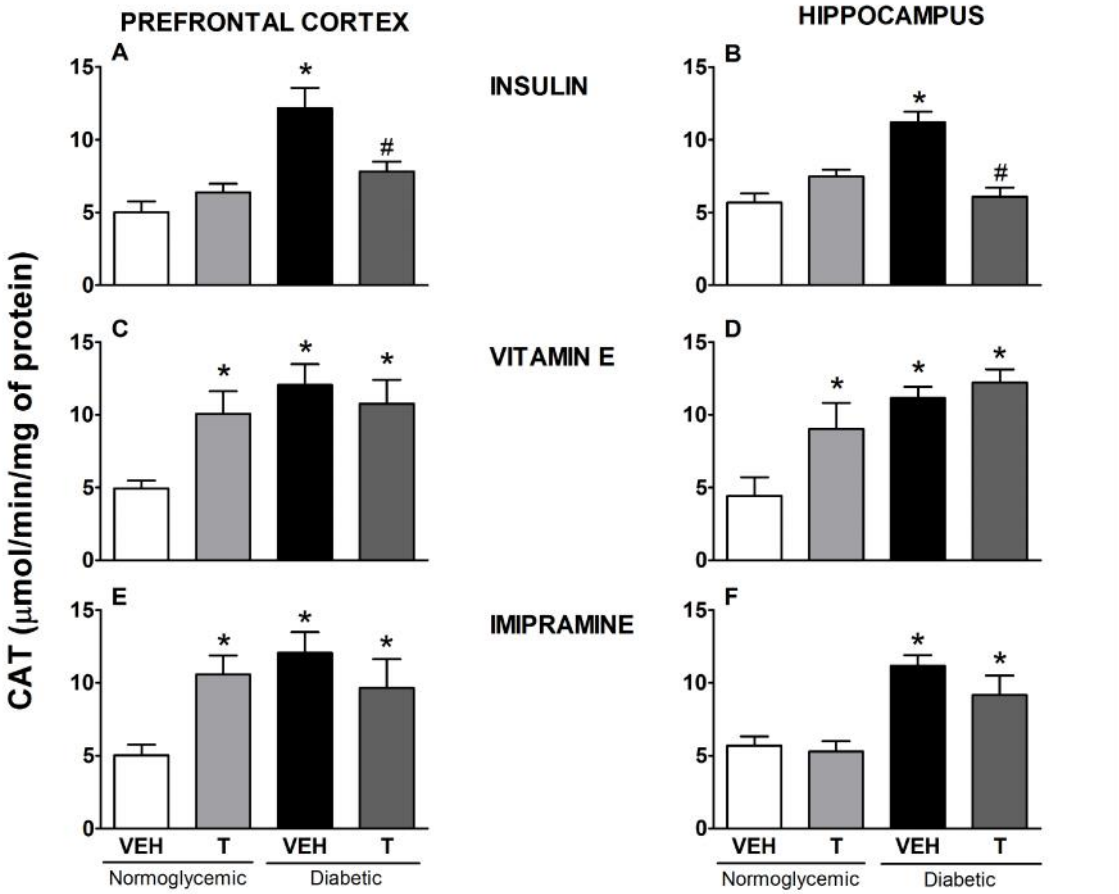

Fig. 8: Effect of treatment (T) with insulin (INS, panels A and B), vitamin E (VIT E, panels C and D), imipramine (IMI, panels E and F) or their respective vehicle (VEH) on the CAT activity (mmol/min/mg of protein) in prefrontal cortex and hippocampus from normoglycemic and diabetic animals. Results are expressed as mean ± SEM, n = 6-9. $^{*}p < 0.05$ compared to the NGL/VEH, $^{\#}p < 0.05$ compared to the DBT/VEH.

3.5.Discussion:

The main finding of the current study is that drugs that caused an improvement in oxidative stress parameters in PFC and HIP induced an antidepressant-like effect in DBT rats when evaluated in the FST, reinforcing

that oxidative stress may have an important role on physiopathology of depression associated to diabetes.

The FST is widely used animal model for screening potential antidepressant drugs. This test is based on the observation that rats, when forced to swim in a restricted space during the test session, eventually ceases to struggle, surrendering themselves to the experimental conditions. This behavioral despair condition is considered to be a depression-like state and is used to evaluate various antidepressant drugs [36]. As expected, the current study demonstrated that NGL animals present this behavioral despair or depressive-like behavior by increasing in the immobility time. According to previous results [37,43,44], our data show that DBT rats, when evaluated in the FST, exhibited a more pronounced depressive-like behavior when compared to NGL animals (Fig. 1, 3, 4). It is important to highlight that first immobility latency, which is the time elapsed between the introduction of the animal in the water until it is completely immobile for the first time [45], corroborates with the results described above, showing that DBT group presented lower first immobility latency when compared to the control NGL group. This behavior, together with the total duration of immobility, reflects depressive-like behavior in animals. Additionally, when evaluated in the open field test, DBT rats did not exhibit any alteration in the locomotor activity when compared to NGL rats (Table 1).

Importantly and unlike the studies conducted by Wayhs and colleagues [37,46], pilot studies conducted in our laboratory showed no change in the time of immobility when DBT animals were evaluated in FST on the 21th day after induction of diabetes by STZ injection (data not shown). However, according to other studies [43,44], we observed a significant effect on the behavioral

responses related to depression on the 28th day after diabetes induction by STZ. Thus, in all experiments conducted in the present study the animals were evaluated 28 days after diabetes induction with STZ.

As a model for type 1 diabetes, it is well established that the administration of STZ results in the pancreatic beta cells toxicity with emergence of clinical signs of diabetes within 2-4 days [47,48]. Thus, the consequent action of STZ in β cells is accompanied by characteristic alterations, such as increase of blood glucose levels, reduction of body weight gain and increase of plasma glycated hemoglobin (HbA1) levels (Fig. 1, 2, 3, 4). It is important to highlight that the levels of glucose and HbA1 are complementary for assessing the control of diabetes, since the results of HbA1 reflects the average blood glucose in the range of one to three months prior to collection, while blood glucose level only at the time of collection of the blood sample [49.50].

Overall, many diabetic complications are caused by prolonged exposure to high glucose levels, particularly in those cells that failure to down-regulate their uptake of glucose when extracellular glucose concentrations are elevated [51,52]. The mechanisms underlying hyperglycemia-induced tissue damage have in common a single pathway, an increase of oxidative stress [17,37,53,54].

In that context, our data show that DBT animals presented an increased oxidative stress in PFC as well as in HIP, as evidenced by the increase of lipid peroxidation levels (Fig. 5) and also of superoxide dismutase (SOD) and catalase (CAT) activities (Fig. 6, 7). Moreover, it was observed a significant decrease of reduced glutathione (GSH) levels (Fig. 8) in these brain areas. It is known that HIP is one of several limbic structures that have been implicated in

depression. In addition, the HIP has connections with the PFC, region that is more directly involved in emotion and cognition and thereby contributes to other major symptoms of depression [55,56].

Supporting our data, it was recently reported a significantly increase in DNA damage in PFC and HIP from DBT rats [46]. It is important to highlight that brain is particularly prone to damage by reactive species of oxygen/nitrogen (ROS/RNS), since this organ requires high oxygen consumption and there is abundance of redox-active metals and large amount of oxidisable polyunsaturated fatty acids and catecholamines, accompanied by a relative deficit in antioxidant systems [57,58]. Evidences show that, as occurs in other diabetic complications, the imbalance between ROS/RNS and antioxidant enzymes also appears to play an important role in the pathogenesis of depression [59,60]. Besides, areas such as HIP and PFC are affected by this imbalance, which allow suggest that increased oxidative stress in these brain areas can be directly related to the development of depression [61-65].

In order to investigate that the increased oxidative stress and a more pronounced depressive-like behavior can be related to hyperglycemic state and not due to a nonspecific effect induced by STZ injection, we firstly investigated the effect of prolonged treatment with INS over depressive-like behavior and oxidative stress parameters in both NGL and DBT rats. As expected, when compared to untreated DBT, INS-treated DBT animals presented a significant reduction in blood glucose levels and in the percentage of HbA1 (Fig. 1, 2). Moreover, the reduced weight gain observed in DBT animals was prevented by INS treatment, suggesting an improvement of general health. Additionally, INS treatment also prevented the more pronounced depressive-like behavior of DBT

animals without changing the depressive-like behavior of NGL animals. Here it is interesting to note that while the glucose levels in NGL animals was reduced after prolonged INS treatment, the percentage of HbA1 was unchanged (Fig. 2). Further, the immobility time and latency of the first immobility of NGL animals treated with INS was not changed when compared to vehicle-treated NGL animals. In contrast, Park and collaborators (2012) [66] observed an increase in the immobility time in mice made hypoglycemic by INS administration. However, this behavioral effect was observed only 24 hours after acute injection of INS (0.8 U/kg, i.p.) being this depressive-like behavior restored after 48 hours.

The antidepressant-like effect induced by INS treatment seems to be dependent of the diabetic condition. This specific antidepressant-like effect can be observed by increasing in the immobility time as well as decreasing in the latency of first immobility (Fig. 1). Reinforcing our results, it was shown that INS is able to induce an antidepressant-like effect in DBT mice submitted to another depression test, the tail suspension test [67]. Others beneficial effects of INS treatment have also been shown when other diabetic complications were evaluated, such as neuropathic pain [68] and diabetic ketoacidosis [69]. Moreover, a neurotrophic role for insulin in the human brain has been proposed [70,71]. Despite the evidence in favor of our results, Wayhs and colleagues (2010) [37] did not observe any improvement in depressive-like behavior in DBT rats treated with INS and exposed to the FST. Probably this effect differs from that observed in the present study due to different INS doses and regimen of treatment.

Interestingly, the antidepressant-like effect of INS treatment in DBT rats was accompanied by prevention of all oxidative stress parameters in PFC and HIP

(Fig. 5, 6, 7, 8). Accordingly, INS treatment induced a significant reduction of brain mitochondrial alterations isolated from STZ-induced diabetic animals, suggesting that besides its well-known significant effect over glycemic control maintenance, this treatment has also an important role on oxidative stress attenuation [72]. Furthermore, the beneficial effects of INS treatment in DBT rats may be due to additional neuroprotective property, since it was observed previously that INS treatment in DBT rats increased the hippocampal cell proliferation and decreased corticosterone levels, two characteristics also related to the antidepressant-like effect [67]. In our study, this neuroprotective effect of INS treatment was evidenced by the increase of GSH levels and reduction of SOD activity in PFC from NGL animals (Fig. 6, 7). Many evidences have also showed that INS as well as insulin-like growth factors may exert important roles in neural development and synaptic plasticity [73,74]. Thus, it seems that not just the hyperglycemia, but also the absence of insulin can be involved in the oxidative stress and depression related to the diabetes.

Next, it was investigated the effect of prolonged treatment with a well-established antioxidant compound VIT E on depressive-like behavior and oxidative stress in PFC and HIP. It was observed for the first time that VIT E treatment exerted an antidepressant-like effect in DBT rats (Fig. 3). This effect seems to be a treatment-specific effect since VIT E did not alter locomotor activity (Table 1). The absence of the antidepressant-like effect in NGL animals can be due to the duration of treatment, dose used and even due to the unknown concentration of α-tocopherol, which is the most active and abundant form of VIT E acting in preventing the propagation of free radical reactions in membranes and lipoproteins [75]. In that context, the long-term treatment (28

days) of NGL mice with α-tocopherol in the dose of the 10 mg/kg (p.o.), but not 30 or 100 mg/Kg, reduced the immobility time in the FST [30].

Although treatment with VIT E has not changed the hyperglycemic condition of the DBT animals, this treatment prevented the reduced weight gain of DBT animals, indicative of general health condition improvement (Fig. 3). Regarding to hyperglycemic condition, studies have shown that VIT E treatment may exert a significant blood glucose reduction [33,76]. In that context, it was observed a reduction in hyperglycemia after 10 weeks of continuous treatment with 400-500 IU/kg/day of VIT E [76]. In another study, a significant reduction in blood sugar was obtained after 5 weeks of treatment with VIT E, but when this treatment was in combination with other antioxidant, selenium [33]. In our study the animals were treated for 4 weeks with the VIT E in the dose of 300 mg/kg (p.o.) which can justify the absence of a reduction in the blood sugar.

The antidepressant-like effect induced by VIT E can be also associated with a significant reduction on oxidative stress parameters in PFC and HIP, because the treatment with VIT E was able to prevent the increased lipid peroxidation and the reduced GSH levels (Fig. 5, 6). Since VIT E is chain breaking lipid soluble, which especially protects biological membranes from lipid peroxidation [77], its antioxidant property had already been reported previously in both NGL and DBT animals [30,33,78,79]. It is also well established by the literature that VIT E has a pivotal role on normal neurological function [80]. In this respect and according our data, the prolonged treatment with VIT E normalized the GSH content and reduced the lipid peroxidation in the PFC and HIP of DBT animals, in which it was related to better performance on learning and memory processes [81]. It is also important to point out that the prolonged and

continuous treatment with antioxidant compounds may be promising for inducing improvement of various complications of diabetes, such as depression (current study), memory [82], skin damage [83] and cardiomyopathy [84].

Our data did not show a beneficial effect of the VIT E treatment in preventing the increased SOD and CAT activity in PFC and HIP from DBT animals (Fig 7, 8). It is interesting to note that VIT E was also not able to prevent elevated CAT activity in HIP from rats during status epilepticus induced by pilocarpine injection [85]. As observed in our study with NGL animals, the authors showed that VIT E elevated the CAT activity in HIP *per se*.

Finally, we tested the effect of prolonged treatment with imipramine (IMI), used as a positive control to the antidepressant-like effect, on behavioral response evaluated in FST and on oxidative stress parameters in PFC and HIP. As expected, and different from the observed with VIT E and INS treatments, IMI treatment induced an antidepressant-like behavior in NGL besides DBT animals (Fig. 4). It is noteworthy that exactly as occurred after VIT E and INS treatments in DBT animals, IMI treatment also induced a not ideal antidepressant-like effect, because the immobility time of the treated DBT animals did not differ of immobility time from vehicle-treated NGL animals. However, even been a partial antidepressant-like effect, an improvement in the evaluated behavioral response was clearly observed after VIT E, INS and IMI treatments in DBT animals.

Despite the IMI treatment decrease the number of crossings in the open field test in NGL and DBT rats suggesting a decreased locomotor activity (Table 1), the antidepressant-like effect seems not to be due to an unspecific effect, since

all IMI-treated rats had a significant reduction of immobility time when evaluated in FST (Fig. 4).

Even though IMI treatment had not improved the high blood glucose levels or the reduced weight gain observed in DBT rats, this treatment ameliorated some oxidative stress parameters in PFC and HIP, such as the increased LOOH content, the decreased GSH levels, as well the increased SOD activity (Fig. 5, 6, 7, 8). In addition and in according to our findings, IMI-treated NGL animals exhibited an increase in CAT activity in PFC, but not in HIP, and any change in the SOD activity [86]. Supporting our findings, consistent evidence for the antioxidant effects of antidepressant drugs have been previously shown in preclinical studies [86,87] and also in clinical studies [27,88,89], suggesting that this antioxidant property may contribute to their therapeutics effects.

It is important to highlight that in our study all different treatments prevented (INS and VIT E) or reversed (IMI) the increased GSH content in PFC and HIP. The mechanism responsible for the increase in GSH content may be related to (i) a direct antioxidant effect, in which the drugs could act as a scavenger of reactive species to preserve GSH content, or (ii) an indirect antioxidant effect, in which the drugs might induce the synthesis of GSH and reduce its degradation through mechanisms that are not yet understood. Thus, further studies are needed to confirm these possibilities. Regarding SOD activity, except for treatment with VIT E, treatment with INS and IMI were able to prevent and reverse, respectively, the increased SOD activity in PFC and HIP from DBT rats. Regarding to CAT activity, only INS treatment was able to prevent its increased activity in PFC and HIP from DBT rats. These enzymes are important parts of the enzymatic antioxidant defense system. SOD is the first line of

defense against reactive species and responsible for catalyzing O2•-, which is highly reactive and toxic, to H_2O_2 (hydrogen peroxide), which is less reactive and toxic [90]. The CAT catalyzes chemical reactions in which the substrate can act as a reducer and an oxidizer. It catalyzes the conversion of H_2O_2 into water and oxygen molecule. Although these enzymes are potent antioxidant defenses, its physiological role appears to be ambiguous because this can lead to an increase in oxidative stress through excessive H_2O_2 formation by SOD, especially if CAT activity is impaired [61]. In our study, the activity of both enzymes, SOD and CAT, were increased in DBT animals, suggesting that organism's defense mechanisms are active in minimizing the increase of oxidative stress. Corroborating our findings, it was demonstrated an elevated CAT and SOD activity in plasma from patients with major depression. Moreover, they observed a significantly positive correlation between the SOD activity and the severity of depression [88].

Taken together, our data demonstrate that INS, VIT E and IMI induce a antidepressant-like effect that can be due to their ability to improve oxidative stress parameters in the PFC and HIP. It is important to highlight that treatment with INS was the most effective in preventing, in DBT rats, parameters related to oxidative stress in these brain areas, reinforcing the importance of having a rigid glycemic control. Since all treatments used in the current study were not able to induce a complete reversal of the depressive-like state of DBT animals, the oxidative stress seems not to be the unique factor involved in the pathophysiology that relate depression to diabetes. Further studies are needed to be conducted to better understand the pathophysiological mechanisms that link depression to diabetes.

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4.0. CONSIDERAÇÕES FINAIS

Em conjunto, nossos dados demonstram que o tratamento com INS, VIT E e IMI induziu um efeito do tipo antidepressivo que pode ser devido à capacidade de melhorar os parâmetros de estresse oxidativo no CPF e HIP. É importante destacar que o tratamento com INS foi o mais eficaz na prevenção, em ratos diabéticos, de parâmetros relacionados ao estresse oxidativo nestas áreas do encéfalo reforçando a importância de se ter um controle glicêmico rígido. Uma vez que todos os tratamentos usados no presente estudo não foram capazes de induzir uma reversão completa do estado do tipo depressivo em animais diabéticos, o stress oxidativo parece não ser o único fator envolvido na fisiopatologia da depressão relacionada ao diabetes. Assim, mais estudos são necessários para compreender melhor os mecanismos fisiopatológicos que relacionam a depressão ao diabetes.

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