FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
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DISSERTAÇÃO DE MESTRADO

EFEITO PROTETOR DA 7-CLORO-4- (FENILSELANIL) QUINOLINA EM UM MODELO "TIPO DIABETES" INDUZIDO POR DIETA HIPERGLICÍDICA EM *Drosophila melanogaster*

DAIANE DE AQUINO SILVA

Uruguaiana Rs/Brasil
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Orientadora: Profa. Dra. Marina Prigol

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Dissertação defendida e aprovada em: 25/02/2018

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DEDICATÓRIA

Dedico esse trabalho a minha amada mãezinha (*in memorian*).
Sinto sua falta, mamãe! Parece que meu peito arde todo os dias ao acordar.
Acho que é saudade; não sei bem explicar. Mas desde a hora que você se foi
tudo se tornou mais cinzento, sem graça, vazio.
A dor da tua falta é imensa.

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“Sorte é o nome que o vagabundo dá ao esforço que ele não faz”.

Leandro Karnal

RESUMO

Dissertação de Mestrado

Programa de Pós-Graduação em Bioquímica/ Universidade Federal do Pampa

EFEITO PROTETOR DA 7-CLORO-4- (FENILSELANIL) QUINOLINA EM UM MODELO "TIPO DIABETES" INDUZIDO POR DIETA HIPERGLICÍDICA EM *Drosophila melanogaster.*

Daiane de Aquino Silva

Orientadora: Profa. Dra. Marina Prigol

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Local e Data da defesa: Uruguaiana, 25 de fevereiro de 2019

A dieta é um componente chave para o desenvolvimento e a longevidade dos organismos. Neste cenário uma atenção especial tem sido dada aos carboidratos, já que dietas ricas em açúcar têm sido ligadas a alterações metabólicas como obesidade e resistência à insulina, que são importantes características do diabetes melittus tipo 2. O objetivo desse trabalho foi utilizar a mosca da fruta *Drosophila melanogaster* para avaliar os efeitos causados pelo consumo de uma dieta hiperglicídica (DH) e avaliar o efeito protetor do composto 7-cloro-4- (fenilselanil) quinolina (4-PSQ). As moscas adultas foram divididas em oito grupos de 50 moscas cada: (1) Dieta regular (DR), (2) DR + 4-PSQ (25 µM), (3) DH 5%, (4) DH 10% , (5) DH 30% , (6) DH 5% + 4-PSQ (25 µM), (7) DH 10% + 4-PSQ (25 µM) e (8) DH 30% + 4-PSQ (25 µM). Moscas foram expostas a uma dieta contendo sacarose e / ou 4-PSQ por 10 dias, de acordo com cada grupo. No final do tratamento avaliou-se a taxa de sobrevivência, a longevidade, a taxa de eclosão, a ingestão alimentar, os níveis de glicose e triglicérides, assim como, alguns marcadores de estresse oxidativo, tais como as substâncias reativas ao ácido tiobarbitúrico (TBARS), a atividade das enzimas, superóxido dismutase (SOD) e catalase (CAT), os níveis de tióis proteicos (PSH) e não proteicos (NPSH) e os ensaios de viabilidade celular: Resazurina e MTT. Observou-se que o consumo de dietas DH foi associado a menor sobrevida da mosca, menor longevidade e aumento dos níveis de glicose, triglicerídeos, TBARS e aumento das atividades das enzimas, SOD e CAT. O tratamento com 4-PSQ aumentou a saciedade das moscas, aumentou a sobrevida, reduziu os níveis de glicose, triglicerídeos e TBARS, aumentou a eclosão, assim como, normalizou a atividade das enzimas, SOD e CAT. Esses experimentos sugerem que o 4-PSQ apresentou um potencial efeito antioxidante e proporcionou uma maior saciedade, atenuando os efeitos do consumo elevado de DH em um modelo tipo diabetes, induzido por dieta em *Drosophila melanogaster.*

Palavras-Chave: Selênio, quinolina glicose, estresse oxidativo, sacarose, diabetes, *D. melanogaster*

ABSTRACT

Dissertation of Master

Program of Post-Graduation in Biochemistry- Federal University of Pampa

PROTECTIVE EFFECT OF 7-CHLORO-4- (PHENYLSELANYL) QUINOLINE IN "DIABETIC-LIKE" MODEL INDUCED PER HIPERGLICIDIC DIET IN *Drosophila melanogaster*

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Co Advisor Drº. Gustavo Petri Guerra

Site and Date of Defence: Uruguaiana, february 25 th, 2019

Diet is a key component for the development and longevity of organisms. In this scenario, special attention has been given to carbohydrates, since sugar-rich diets have been linked to metabolic alterations such as obesity and insulin resistance, which are important characteristics of type 2 diabetes mellitus. The objective of this work was to use the fruit fly *Drosophila melanogaster* to evaluate the effects of hyperglycidic diet (HD) and to evaluate the protective effect of 7-chloro-4- (phenylselenyl) quinoline (4-PSQ). Adult flies were divided into eight groups of 50 flies each: (1) RD, (2) RD + 4-PSQ (25 µM), (3) regular diet (HD) 5%, (4) HD 10%, (5) HD 30% (6) HD 5% + 4-PSQ (25 µM), (7) HD 10% + 4-PSQ (25 µM) and (8) HD 30% + 4-PSQ (25 µM). Flies were exposed to a diet containing sucrose and / or 4-PSQ for 10 days, according to each group. At the end of treatment the survival rate, longevity, hatch rate, food intake, glucose and triglyceride levels, as well as some markers of oxidative stress, such as thiobarbituric acid reactive substances (TBARS), enzyme activity, superoxide dismutase (SOD) and catalase (CAT), protein thiol (PSH) and non-protein levels (NPSH) and cell viability assays: Resazurin and MTT. It was observed that the consumption of DH diets was associated with lower survival of the fly, lower longevity and increased levels of glucose, triglycerides, TBARS and increased activities of enzymes, SOD and CAT. Treatment with 4-PSQ increased the satiety of flies, increased survival, reduced glucose, triglyceride and TBARS levels, increased hatching, as well as normalized enzyme activity, SOD and CAT. These experiments suggest that 4-PSQ had a potential antioxidant effect and provided greater satiety by attenuating the effects of high HD consumption on a diet-induced diabetes-like model in *Drosophila melanogaster*.

Keywords: Selenium, quinoline, glucose, oxidative stress, sucrose, diabetes, *D. melanogaster*

LISTA DE FIGURAS

Revisão Bibliográfica

LISTA DE ABREVIATURA E SIGLAS

Revisão Bibliográfica

7-cloro-4-(fenilselanil) quinolina (4-PSQ)

Ácido Hipocloroso (HCLO)

Ácidos graxos livres (AGL)

Catalase (CAT)

Células Produtoras de Insulina (CPI)

Diabetes mellitus 2 (DM2)

Drosophila melanogaster (D. melanogaster)

Espécies reativas ao ácido tiobarbitúrico (TBARS)

Espécies reativas de nitrogênio (ERN)

Espécies reativas de oxigênio (EROS)

Flavina adenina reduzida ($FADH_2$)

Glicação avançada (AGEs)

Hormônio adipocinético (AKH)

Nicotinamida adenina reduzida (NADH)

Organização mundial da saúde (OMS)

Óxido Nítrico (NO)

Peptídeo semelhante à insulina (DILPS)

Peroxinitrito (OONO-)

Proteína C kinase C (PKC)

Radical Alcoxil (RO)

Radical Ânion Superóxido (O_2-)

Radical Hidroxil (HO)

Radical Peroxil (ROO)

Sociedade Brasileira de Diabetes (SBD)

Superóxido Dismutase (SOD)

Tióis não protéicos (NPSH)

Tióis protéicos (PSH)

Triglicerídeos (TGs)

Viabilidade celular quantificada pela redução do tetrazólio a formazan (MTT)

World Health Organization (WHO)

Sumário

APRESENTAÇÃO

No item **INTRODUÇÃO E REVISÃO BIOBLIOGRÁFICA** consta uma revisão da literatura sobre os temas trabalhados nesta dissertação.

A metodologia realizada e os resultados obtidos que compõem esta dissertação estão apresentados sob a forma de artigo, os quais se encontram no item **MANUSCRITO**. Neste constam as seções: Introdução, Materiais e Métodos, Resultados, Discussão, Conclusão e Referências Bibliográficas.

Os itens **CONCLUSÃO** e **PERSPECTIVAS FUTURAS** encontrada no final desta dissertação, apresenta Interpretações e comentários gerais sobre o artigo científico incluído neste trabalho.

As **REFERÊNCIAS BIBLIOGRÁFICAS** dispostas no final referem-se somente às citações que aparecem nos itens **INTRODUÇÃO E REVISÃO BIBLIOGRÁFICA** dessa dissertação.

INTRODUÇÃO

O aumento na longevidade da população associado às alterações no estilo de vida, sobretudo as mudanças no padrão de alimentação, contribuem para o aumento do perfil de risco para doenças crônicas, como o diabetes mellitus (DM). A prevalência dessa doença tem aumentado consideravelmente, sendo considerada pela Organização Mundial da Saúde (OMS) como um grave problema de saúde pública (OMS, 2018).

Segundo a Sociedade Brasileira de Diabetes (SBD, 2018), atualmente, existem mais de 12 milhões de portadores da doença. Os diabéticos tipo 2 (DM2) representam 90% a 95% dos casos, sendo que a grande maioria apresenta sobrepeso ou obesidade. É importante ressaltar que o diabetes apresenta alta morbimortalidade, sendo uma das principais causas de mortalidade, insuficiência renal, amputação de membros inferiores, cegueira e de doença cardiovascular, incluindo doenças coronarianas e acidentes vasculares encefálicos.

Nas últimas décadas, tem havido um interesse crescente nos papéis do consumo excessivo de carboidratos no desenvolvimento de distúrbios metabólicos. Além dos modelos de mamíferos, novos modelos não vertebrados, como vermes e insetos, foram introduzidos para identificar os mecanismos moleculares e as vias genéticas envolvidas na iniciação e progressão de diferentes doenças metabólicas (ROVENKO et al., 2015, MUSSELMAN & KÜHNLEIN 2018, OWUSU -ANSAH & PERRIMON, 2014).

A mosca da fruta *Drosophila melanogaster* é um modelo bastante viável para o estudo da obesidade e doenças metabólicas por várias razões. Primeiro, as moscas contêm tecidos, órgãos e sistemas análogos as vias envolvidas na obesidade humana e doenças metabólicas associadas. MUSSELMAN et al., (2011) comprovou que a *Drosophila* desenvolve a obesidade e suas complicações associadas, com sobrecarga calórica, semelhante aos seres humanos. Além disso, a maioria dos genes e famílias de genes conhecidos por funcionarem na doença metabólica são conservados entre moscas e humanos (REITER, 2001).

Considerando a alta incidência dessa condição metabólica do DM2, bem como as significativas taxas de morbidade e morbimortalidade que a doença

traz para a população afetada, reforça-se a necessidade de melhor compreender os mecanismos e fatores relacionados às suas possíveis complicações e ao seu controle. Neste contexto, existe um grande interesse em utilizar a *Drosophila melanogaster* no estudo de novos compostos para o tratamento de distúrbios associados ao DM2.

O interesse em compostos orgânicos de selênio tem intensificado devido ao seu potencial farmacológico. Um composto de bastante interesse atualmente é o 7-cloro-4-(fenilselanil) quinolina (4-PSQ), um organoselenio com anel quinolina que tem demonstrado potencial terapêutico e farmacológico. Descrito pela primeira vez por SAVENAGO et al. (2013), o composto 4-PSQ tornou-se alvo de interesse em diversos estudos que verificaram suas propriedades como atividade antioxidante, anti-inflamatória, antinociptiva, ansiolítica e anticonvulsivante (WILHELM et al., 2014; PINZ et al., 2016; REIS et al., 2014) em modelos animais. Mais recentemente, COUTO et al.,(2018) constatou efeitos neuroprotetores do 4-PSQ em um modelo de doença de Parkinson induzido por rotenona em *Drosophila melanogaster*, porém ainda não se tem dados sobre a atuação desse composto em modelo de doença metabólica, especialmente o DM2.

JUSTIFICATIVA

Na busca de uma estratégia para a intervenção dos danos causados pelo excesso de carboidratos e DM 2, considerando a prevalência da doença e os graves efeitos que ela causa, é necessário explorar potenciais novos fármacos para o enfrentamento desse distúrbio. A quinolina e seus derivados tem apresentado um vasto campo de atuação e variadas atividades biológicas, e tem sido constantemente relatadas com atividades significativas no tratamento de diversas patologias. Portanto, esse trabalho visou explorar os efeitos do alto consumo de sacarose nas respostas fenotípicas compatíveis com DM 2, bem como os possíveis danos oxidativos e efeito terapêutico da composição do 4-PSQ em D*rosophila melanogaster.*

OBJETIVO GERAL

Investigar um possível efeito protetor do composto 7-cloro-4-(fenilselanil) quinolina (4-PSQ) sobre alterações metabólicas em um modelo “tipo diabetes” induzido por dieta hiperglicidica em *Drosophila melanogaster*.

OBJETIVOS ESPECÍFICOS

Avaliar a taxa de sobrevivência e longevidade de *Drosophila. melanogaster* após exposição à dieta hiperglicidica e tratamento com 4-PSQ.

Analisar possíveis alterações nos níveis de glicose e triglicerídeos em *Drosophila melanogaster* após exposição crônica à dieta hiperglicidica e tratamento com 4-PSQ.

Quantificar a taxa de eclosão e o percentual de ingestão alimentar de *Drosophila melanogaster* após exposição crônica a dieta hiperglicidica e tratamento com 4-PSQ.

Avaliar o envolvimento do estresse oxidativo após a exposição crônica a dieta hiperglicidica e tratamento com 4-PSQ.

Avaliar a viabilidade celular e mitocondrial de *Drosophila. melanogaster* após exposição crônica a dieta hiperglicidica e tratamento com o 4-PSQ

REVISÃO BIBLIOGRÁFICA

1. Diabetes mellitus (DM)

O Diabetes mellitus (DM) é uma doença metabólica crônica não transmissível de origem multifatorial caracterizada pela elevação permanente dos níveis glicêmicos decorrente da ausência e/ou incapacidade da insulina de exercer sua função fisiológica, gerando uma série de complicações e disfunções de órgãos essenciais (CORRÊA et al.,2017). Classifica-se o diabetes em três principais categorias:

1. Diabetes mellitus Tipo 1 (DM1): decorrente da destruição autoimune das células beta-pancreáticas. Normalmente, apresenta-se com deficiência absoluta de insulina.

2. Diabetes mellitus Tipo 2 (DM2): Está relacionado a diminuição progressiva da secreção insulínica e, é acompanhado, frequentemente, por resistência insulínica. Cerca de 90 a 95% dos portadores da doença são do tipo 2, muito ligado a fatores ambientais e estilo de vida.

3. Diabetes mellitus gestacional (DMG): hiperglicemia detectada pela primeira vez durante a gestação, porém, com níveis glicêmicos sanguíneos que não atingem os critérios diagnósticos para DM.

Outros tipos específicos menos frequentes são descritos, tais como: diabetes por defeitos genéticos da função da célula β, diabetes por defeitos genéticos da ação da insulina, diabetes secundários (a doenças do pâncreas exócrino, a endocrinopatias, induzido por drogas ou produtos químicos, infecções) (SBD, 2018).

Segundo as Diretrizes da Sociedade Brasileira do Diabetes (SBD), (2017-2018) o DM2 é um importante e crescente problema de saúde para todos os países, independentemente do seu grau de desenvolvimento. As consequências humanas, sociais e econômicas são devastadoras: são 4 milhões de mortes por ano relativas ao diabetes e suas complicações (com muitas ocorrências prematuras), o que representa 9% da mortalidade mundial total (CORRÊA et al.,2017).

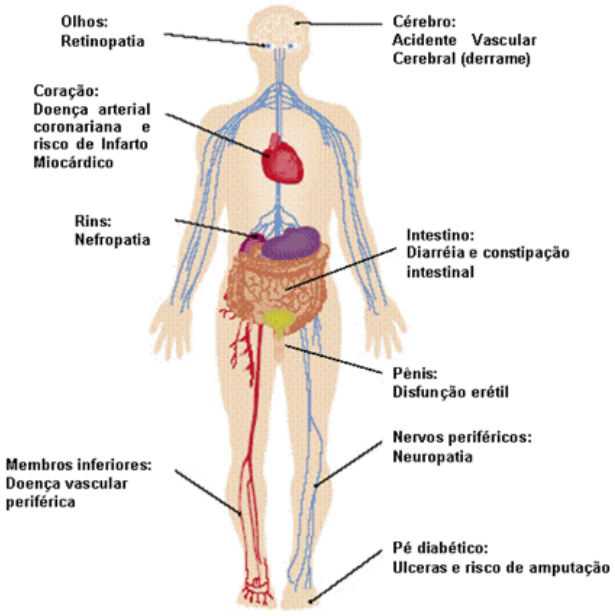
O aumento da prevalência do diabetes está associado a diversos fatores, como: rápida urbanização, transição epidemiológica, transição nutricional, maior frequência de estilo de vida sedentário, maior frequência de

excesso de peso, crescimento e envelhecimento populacional e, também, à maior sobrevida dos indivíduos com diabetes (SBD, 2018) .

A Organização Mundial da Saúde (OMS) estima que a glicemia elevada é o terceiro fator, em importância, da causa de mortalidade prematura, superada apenas por pressão arterial aumentada e uso de tabaco. As estimativas da (OMS, 2018) sobre DM2 sugerem que a doença foi responsável por 1,4 milhão de óbitos em 2011. Apontam ainda que, entre 2010 e 2030, haverá um aumento de 69% no número de adultos com DM2 nos países em desenvolvimento e de 20% nos países desenvolvidos. Até 2025, a expectativa é de 350 milhões de pessoas acometidas pela doença no mundo. Estima se que em 2025, no Brasil, serão 18,5 milhões (OMS,2018; CORRÊA et al.,2017). O DM2 representa 5,2% das causas de mortes no Brasil e, junto com outras doenças crônicas, que atinge especialmente grupos populacionais vulneráveis, como os idosos e aqueles de baixa renda e escolaridade, tornando o assunto de interesse para a saúde pública (ARTILHEIRO et al., 2014)

Tradicionalmente, as complexidades do diabetes são categorizadas como distúrbios microvasculares e macrovasculares, que resultam em retinopatia, nefropatia, neuropatia, doença coronariana, doença cerebrovascular e doença arterial (OMS, 2019) (Figura 1).

Figura 1 Complicações do diabetes



Fonte: SBD,2019 com adaptações

O aparecimento das complicações crônicas está diretamente relacionado com a evolução do diabetes e com o controle metabólico realizado. Posteriormente os danos metabólicos do diabetes podem envolver a insuficiência renal, a amputação de membros inferiores, a cegueira, doenças coronarianas e acidentes vasculares encefálicos. Estas complicações são consideradas as principais responsáveis pela morbidade e mortalidade do DM, o que acarreta perdas importantes na qualidade de vida, além de resultar em altos encargos para os sistemas de saúde (FERNANDES et al., 2019).

2. Relação entre Estresse oxidativo e DM2

O estresse oxidativo é um desequilíbrio entre os agentes oxidantes e antioxidantes, com predominância dos agentes oxidantes do organismo humano e está relacionado ao aparecimento de muitas doenças crônicas (TURK., 2010). As espécies reativas de oxigênio (EROS) são produzidas pelo metabolismo celular, porém quando ocorre o acúmulo de EROS e há a ineficiência na capacidade de remoção do seu excesso no organismo humano há um desequilíbrio que resulta no agravamento de lesões celulares (TURK., 2010).

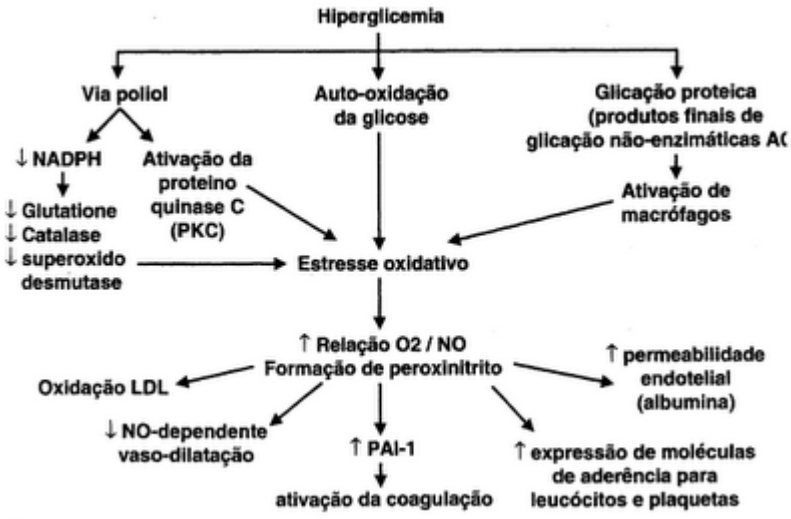
A maioria das moléculas reativas está dividida entre espécies reativas de oxigênio (EROS) e de nitrogênio (ERN). Dentre as mais comuns, estão inclusos o radical ânion superóxido (O_2-) e o óxido nítrico (NO), que dão origem a radicais com grande potencial danoso como o radical hidroxil (HO), radical alcoxil (RO) e peroxil (ROO). Muitos desses radicais são convertidos em moléculas oxidantes, como o peróxido de hidrogênio (H_2O_2), peroxinitrito (OONO-) e ácido hipocloroso (HCLO) (KUNWAR & PRIYADARSINI, 2011).

O ponto inicial, no qual a hiperglicemia altera a função vascular é no desequilibrio entre a biodisponibilidade do NO e o acúmulo de ERO, levando a lesão tecidual. Estas mudanças estão relacionadas ao tempo prolongado de exposição à hiperglicemia, somados a hipertensão arterial, dislipidemia e susceptibilidade genética (BECKMAN et al., 2013). Desse modo, as EROS desempenham papel fundamental no desenvolvimento de doenças crônicas (ZHANG et al., 2018).

Os eventos centrais da fisiopatologia do DM2, a resistência insulínica e a disfunção da célula beta pancreática, ocorrem diante do desequilíbrio de óxido redução, assim como o desenvolvimento das principais complicações da doença (TIWARI et al., 2013). Em um ambiente hiperglicêmico, a maioria das células é capaz de limitar a entrada de glicose, portanto, a concentração de glicose intracelular permanece constante. As células beta pancreáticas também sofrem com maior intensidade os efeitos da hiperglicemia, já que como característica são umas das células que possuem os menores níveis de defesa antioxidantes (TANGVARASITTICHAI, 2015).

Muitas vias bioquímicas são afetadas por esta condição, sendo que elas convergem para a produção de espécies reativas e a instalação do estresse oxidativo. Essas vias incluem o aumento do fluxo de glicose e outros açúcares pela via dos polióis, aumento da formação intracelular de produtos finais de glicação avançada (AGEs), ativação das isoformas da proteína kinase C (PKC) e aumento da atividade da via das hexosaminas (BROWNLEE, 2001, 2005) (Figura 2).

Figura 2 Via dos poliois



Fonte: arquivo próprio adaptado de Fernandes et al., 2019

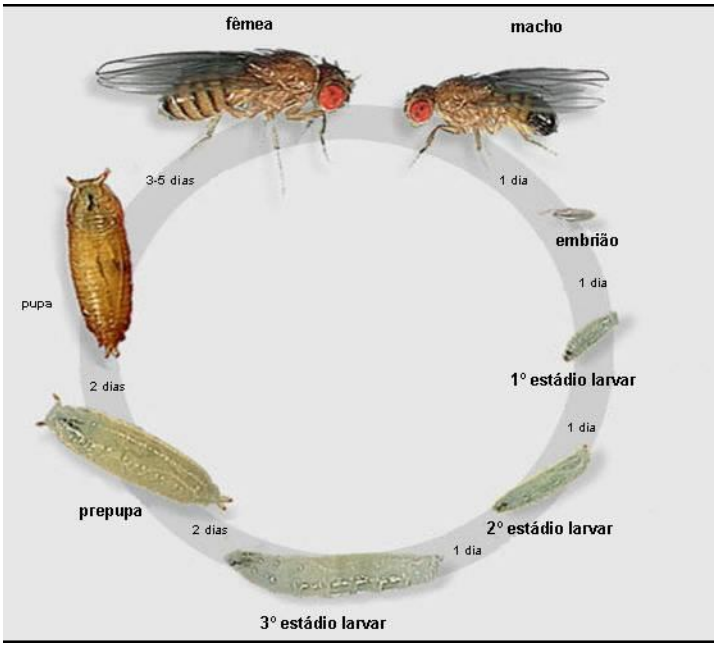
Segundo TANGVARASITTICHAI (2015), o oxidante mais poderoso formado em sistemas biológicos é o radical hidroxila que pode atacar qualquer molécula biológica. A etapa de iniciação da peroxidação lipídica ocorre quando os radicais hidroxila atacam os ácidos graxos poliinsaturados, causando a oxidação dos ácidos graxos poliinsaturados de radicais livres em sistemas biológicos. A peroxidação lipídica é autocatalítica e a produção de radicais

livres mediados por ácidos graxos poliinsaturados está presente no processo de destruição e degradação de membranas celulares envolvidas no transtorno metabólico associado ao DM2 (TANGVARASITTICHAI, 2015). Em excesso de dietas ricas em carboidratos, a glicose excessiva ocorre e uma grande quantidade é metabolizada na glicólise levando ao aumento de NADH e $FADH_2$, na cadeia de transporte de elétrons de mitocôndrias e aumento da produção de superóxido (SALAMEH et al., 2019). A interação entre nutrição e estado metabólico tem implicações importantes para a saúde. A ingestão de antioxidantes está sendo considerada como um fator de proteção contra o dano oxidativo celular e complicações metabólicas relacionadas ao DM2 (GALARREGUI et al.,2018).

3. A *Drosophila melanogaster* como um modelo para indução do Diabetes Tipo 2

A mosca da fruta *Drosophila melanogaster* (Figura 3) esta na vanguarda da biologia, seu crescimento e reprodução rápida, e o fato de ser barato e fácil de manter em laboratório são características que fazem a *Drosophila melanogaster* um sistema modelo viável para abordar questões biológicas inovadoras, incluindo as de saúde humana (GRAHAM & PICK et al., 2017).

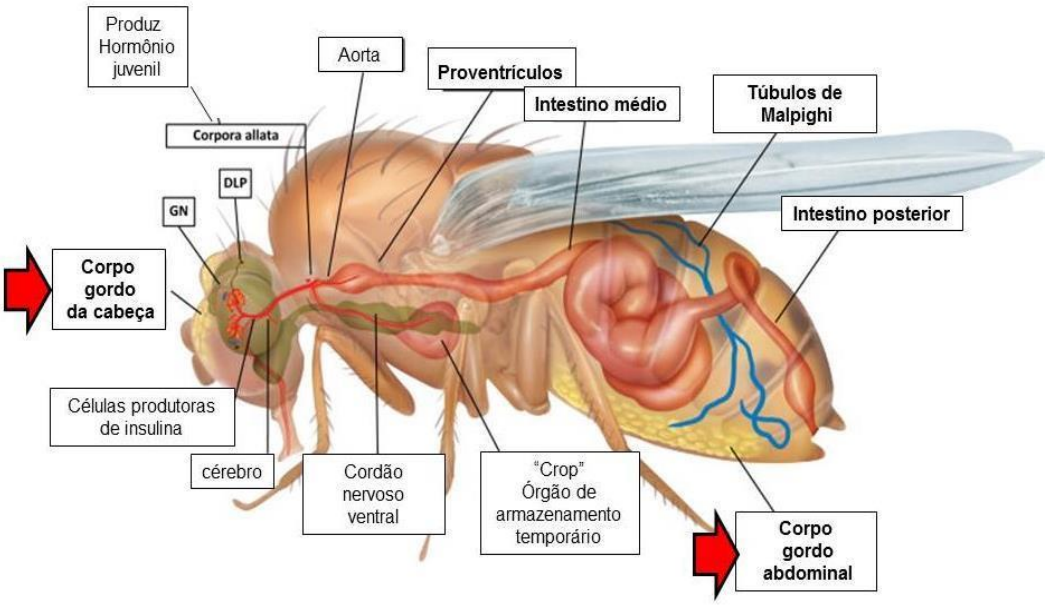
Figura 3 Ciclo de vida de *Drosophila melanogaster*



Fonte: Adaptado de Morris et al.,2012

Os mecanismos que mantêm o equilíbrio entre as formas de energia armazenada e circulante parecem ser amplamente compartilhados entre os animais (GRAHAM & PICK et al., 2017) (Figura 4).

Figura 4 Modelo de *Drosophila melanogaster* representando a localização do corpo de gordura no animal



Fonte: Paula et., 2017, adaptado de Tatar et al. (2014)

Nos mamíferos, quando a energia é abundante (após a ingestão), o excesso de glicose é armazenado como glicogênio, principalmente no músculo esquelético e no fígado. Os ácidos graxos livres (AGL) são armazenados como triglicerídeos (TGs) predominantemente no tecido adiposo, e também no fígado. Quando a energia é insuficiente (após jejum ou exercício), essas reservas são mobilizadas e liberadas para uso (PASCO & LÉOPOLD., 2012).

Os organismos vivos podem se adaptar às mudanças ambientais por meio da regulação hormonal e da homeostase metabólica. Os açúcares são utilizados principalmente na produção metabólica de fontes de ATP e carbono. Em insetos, o açúcar no sangue é armazenado como trealose, um dissacarídeo, e mantém a glicose em níveis reduzidos, mas ainda circulantes, na hemolinfa (YASUGi et al., 2017). Na *Drosophila melanogaster* uma dieta rica em açúcar leva a níveis elevados de triglicérides, glicose circulante e resistência à insulina, fenótipo semelhante a mamíferos com DM2 e uma molécula semelhante a glucagon, o hormônio adipocinético (AKH) é liberado

em resposta a baixos níveis de açúcar circulante (SONG et al.,2017). Recentemente HIETAKANGAS & LEMAITRE (2017) relataram que em larvas o hormônio adipocinético (AKH) é estimulado por baixa glicose e trealose. Eles concluíram que a secreção de (AKH) ativa células produtoras de insulina (CPI). A ativação de CPI por AKH aumenta a sinalização periférica de insulina através de um mecanismo que estimula seletivamente a secreção de dILP3. O papel estabelecido por AKH funciona na *Drosophila melanogaster* como um fator endócrino análogo ao glucagon de mamíferos. Isso permitiu o uso de *Drosophila* como uma ferramenta genética para explorar a resistência à insulina induzida por dieta (SONG et al.,2017).

A exposição das moscas á dietas com nutrientes aumentados ou diminuídos provoca a desregulação do seu metabolismo e da sinalização da insulina. Dietas ricas em açúcar aumentam a expressão do peptídeo semelhante à insulina (DILPS) (MUSSELMAN et al.,2011; ÁLVAREZ-RENDÓN et al., 2018). Este aumento inicial na expressão de DILPS é consistente com o que é observado em vertebrados no aumento da resistência à insulina, onde o organismo inicialmente tenta aumentar sua produção de insulina para compensar o excesso de nutrientes ingeridos (SAMUEL & SHULMAN., 2016). Similarmente, em moscas superalimentadas, o corpo adiposo reduz secundariamente sua resposta insulínica a DILPS circulantes e essa diminuição diminui significativamente à medida que a exposição á dieta se prolonga, tornando as moscas completamente resistentes com o passar do tempo. A combinação de ferramentas genéticas fáceis de usar e poderosas faz da *Drosophila* um sistema modelo convincente para investigar doenças metabólicas complexas, como o diabetes (MORRIS et al.,2012).

Como consequência da hiperglicemia e do estresse oxidativo presente no DM2, uma intensificação na busca por compostos naturais ou sintéticos que exibam propriedades antioxidantes e anti-hiperglicêmicas tem sido feita nas últimas décadas (BARBOSA et ., 2008).

4. Compostos quinolínicos e orgânicos de selênio

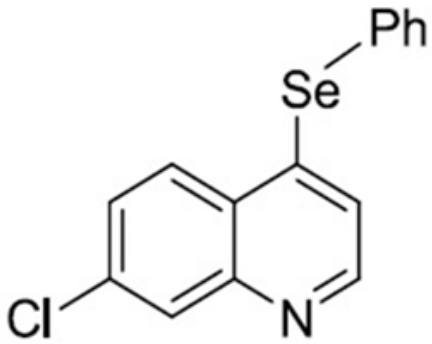
O selênio (Se) é um micronutriente com uso potencial na prevenção e tratamento de doenças (BORTOLATTO, 2017). Os compostos orgânicos de

selênio podem melhorar as defesas celulares naturais contra os agentes oxidantes e podem representar uma nova alternativa terapêutica, para doenças em que o estresse oxidativo está fortemente envolvido (NOGUEIRA, & ROCHA.,2011). Estudos tem demonstrado o efeito farmacológico destes compostos, como por exemplo, atividade antioxidante, anti-inflamatória, antinoceptiva, ansiolítica e anticonvulsivante (WILHELM et al., 2014; PINZ et al.,2016; REIS et al., 2017).

Paralelamente aos compostos orgânicos de selênio estão os derivados de quinolina. A quinolina foi isolada, pela primeira vez, no ano de 1934, quando o químico francês Charles Gerhardt provocou a decomposição de um extrato de quinina, obtido de um arbusto da família das *Rubiáceas*. (MANJUNATHA et al., 2013). Os índios da América do Sul já utilizavam extratos de Rubiáceas no tratamento de doenças infecciosas como a malária, que era comum nas florestas tropicais. Alguns anos após o químico francês Hoogewerff Van Dorp relatou a presença do mesmo núcleo quinolínico em frações de alcatrão de hulha (ACHESON, 1977). As quinolinas são compostos heterocíclicos sintéticos ou naturais com interessantes atividades biológicas (MANJUNATHA et al., 2013).

Um desses compostos vem se destacando, o 7-cloro-4-(fenilselanil) quinolina (4-PSQ) (Figura 5), teve sua capacidade antioxidante demonstrada pela primeira vez por SAVEGNAGO et al. (2013). Neste estudo, os autores demonstraram que o 4-PSQ apresentou a capacidade redutora de íons férrico e agiu inibindo a peroxidação lipídica, indicando uma potencial atividade antioxidante deste composto *in vitro*. Com base nesses resultados, ampliou-se o interesse em aprofundar os estudos em diferentes modelos animais com este composto (WILHELM et al., 2017).

Figura 5 Estrutura química do composto 7-cloro-4-(fenilselanil) quinolina (4-PSQ)



Fonte: Savenagno et al., 2013

Dentre as propriedades farmacológicas relevantes do composto 4-PSQ, ressalta-se, portanto, a ação antioxidante, a qual é de extrema significância no tratamento de doenças crônicas. PINZ et al, (2016), verificou em seu estudo que o 4-PSQ reduziu os níveis de espécies reativas induzidos por óleo de cróton na orelha dos camundongos. SALGUEIRO et al, (2017) demonstraram que o 4-PSQ apresentou efeito antioxidante, dependente da dose usada, em um modelo *in vivo* de *Caenorhabditis elegans*. Os autores observaram que o 4-PSQ interagiu com proteínas tiólicas, que após a oxidação dos grupos sulfidrila é capaz de aumentar a expressão de Superóxido dismutase-3 e Glutationa S-transferase-4, melhorando assim a resposta ao estresse. Mais recentemente, VOGT et al., (2018), comprovou que o composto, na dose de 10 mg/kg, reduziu o estresse oxidativo cerebral induzido pelo nitroprussiato de sódio em camundongos. COUTO et al. (2018) demonstrou em seu estudo um resultado promissor utilizando o 4-PSQ em *Drosophila melanogaster* onde comprovou efeito antioxidante e efeito preventivo exercido pelo composto em um modelo de doença de Parkinson induzido por rotenona.

Em relação à toxicidade, Pinz et al. (2016) evidenciaram que o composto na dose de 25 mg/kg pela via oral não alterou os níveis de ureia e creatinina, não causando toxicidade renal, como também não alterou a atividade das transaminases, não causando toxicidade hepática, nos camundongos.

Adicionalmente, o tratamento com o 4-PSQ na dose de 50 mg/kg, por esta mesma via, também não alterou os níveis de ureia e a atividade das transaminases. Além disso, os parâmetros de estresse oxidativo no cérebro e

no fígado dos camundongos não foram alterados com o tratamento (REIS et al., 2017).

Diante do papel de compostos de selênio com funções antioxidantes reconhecidas por vários pesquisadores como antioxidante. Propomos pela primeira vez a utilização do composto 4-PSQ para o estudo experimental em um modelo mimético a DM 2. Não existem dados na literatura utilizando o 4PSQ em modelo tipo diabetes, por se tratar de um composto de estudo recente, existe ainda muitas possibilidades farmacológicas a serem estudadas devido ao seu diferencial anel quinolinico. Suas propriedades antioxidantes podem ser uteis no tratamento das anormalidades estruturais e funcionais associadas com a exposição prolongada dos tecidos vasculares à hiperglicemia nos indivíduos diabéticos, facilitando a remoção dos radicais livres.

MANUSCRITO

Os resultados que fazem parte dessa dissertação estão apresentados na forma de manuscrito. As seções materiais e métodos, resultados, discussão, conclusão e referências do manuscrito encontram-se no próprio manuscrito.

Posteriormente, serão apresentadas a seção conclusão, perspectivas futuras e referências bibliográficas da dissertação. O manuscrito está disposto segundo as normas da revista Biomedicine & Pharmacotherapy.

7-chloro-4- (phenylselanyl) quinoline co-treatment prevent oxidative stress in diabetic-like phenotypes induced by hyperglycidic diet in *Drosophila melanogaster*

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ABSTRACT

Diet is a key component for the development and longevity of organisms. In this scenario, special attention has been given to carbohydrates, since sugar-rich diets have been linked to metabolic alterations such as obesity and insulin resistance, which are important characteristics of type 2 diabetes mellitus. The objective of this work was to use the fruit fly Drosophila melanogaster to evaluate the effects of hyperglycidic diet (HD) and to evaluate the protective effect of 7-chloro-4- (phenylselanyl) quinoline (4-PSQ). Adult flies were divided into eight groups of 50 flies each: (1) RD, (regular diet) (2) RD + 4-PSQ (25 μM), (3) HD 5%, (4) HD 10%, (5) HD 30% (6) HD 5% + 4-PSQ (25 μM), (7) HD 10% + 4-PSQ (25 μM) and (8) HD 30% + 4-PSQ (25 μM). Flies were exposed to a diet containing sucrose and / or 4-PSQ for 10 days, according to each group. At the end of treatment the survival rate, longevity, hatch rate, food intake, glucose and triglyceride levels, as well as some markers of oxidative stress, such as thiobarbituric acid reactive substances (TBARS), enzyme activity, superoxide dismutase (SOD) and catalase (CAT), protein thiol (PSH) and non-protein levels (NPSH) and cell viability assays: Resazurin and MTT. It was observed that the consumption of DH diets was associated with lower survival of the fly, lower longevity and increased levels of glucose, triglycerides, TBARS and increased activities of enzymes, SOD and CAT. Treatment with 4-PSQ increased the satiety of flies, increased survival, reduced glucose, triglyceride and TBARS levels, increased hatching, as well as normalized enzyme activity, SOD and CAT. These experiments suggest that 4-PSQ had a potential antioxidant effect and provided greater satiety by attenuating the effects of high HD consumption on a diet-induced diabetes-like model in Drosophila melanogaster.

Keywords: Selenium, glucose, oxidative stress, quinolone, sucrose**,** diabetes, *D. melanogaster*

1. INTRODUCTION

Diabetes mellitus (DM) has been identified as metabolic syndrome caused by lack and/or inability of the pancreas to secrete insulin to properly exercise its effects, presenting hyperglycemia among other manifestations. This disease has increased in the number of cases in the population, which in the year 2018 had affected 400 million people around the world (World Health Organization - WHO, 2018).

Over the last few decades, there has been a growing interest in the roles of excessive carbohydrate consumption in the development of metabolic disorders. (ROVENKO et al., 2015). In addition to mammalian models, new non-vertebrate models such as worms and insects were introduced to identify the molecular mechanisms and genetic pathways involved in the initiation and progression of different metabolic diseases (ROVENKO et al., 2015, MUSSELMAN and KÜHNLEIN 2018, OWUSU-ANSAH and PERRIMON, 2014).

The use of *Drosophila melanogaster* in diabetes-like phenotype, was developed by manipulating sucrose concentrations in the diet and described for the first time in the study by MUSSELMAN et al., (2011). According to the literature, it was evidenced that chronic feeding of diets high in carbohydrates and high in fat produces obesity in flies, as in humans, along with a series of pathophysiological complications (ECKER et al., 2017; PAULA et al., 2018). Dietary-induced excess carbohydrate consumption in flies is associated with many of the pathophysiological mechanisms found in humans, including hyperglycemia, insulin resistance, cardiac arrhythmia and fibrosis, reduced longevity (BUESCHER et al., 2013, GARRIDO et al., MUSSELMAN et al. , 2011).

Considering the high incidence of this metabolic condition of DM, as well as the significant diseases and morbimortality rates that the disease brings to the affected population, it has reinforced the need to better understand the mechanisms and factors that are related to its possible complications. In this context, there is great interest in using *Drosophila melanogaster* in the study of new compounds for the treatment of disorders associated with the "diabetes-like" phenotype (ECKER et al., 2017).

Thus, the interest in compounds that could potentially reduce the biological changes associated with hyperglycemia is intense. Selenium is known to be an essential element that plays a crucial role as an integral component of several enzymes with antioxidant properties (NOGUEIRA & ROCHA,2011; BARBOSA et al.,2008). This element due to its physiological antioxidant properties have already demonstrated

potentially beneficial effects against the development of different diseases, including DM, acting on different mechanisms, attenuate the cytotoxic effects of hyperglycemia (BARBOSA et al., 2008; NOGUEIRA & ROCHA, 2004).

Among the new therapeutic proposals is the 7-chloro-4- (phenylalanyl) quinoline (4-PSQ), quinoline derivative with an organoselenium substitute that demonstrated pharmacological potential, first described by SAVENAGO et al., (2013),in this study, the authors demonstrated that the 4-PSQ had a reduced capacity of iron and ions inhibiting lipid peroxidation. In fact, the compound 4-PSQ has become a target of interest in several studies that verified its properties such as antioxidant activity, anti-inflammatory, antinociceptive, anxiolytic and anticonvulsant (WILHELM et al., 2014; PINZ et al.,2016; REIS et al., 2017) in animal models. COUTO et al., 2018 demonstrated in their study a promising result using 4-PSQ in *Drosophila melanogaster* where it demonstrated antioxidant effect and preventive effect exerted by the compound in a model of Parkinson's disease induced by rotenone.

Therefore, the aim of this work is to explore the effects of high sucrose consumption on phenotypic responses consistent with DM, as well as the potential oxidative damages and therapeutic effect of 4-PSQ compound.

2. MATERIALS AND METHODS

2.1 Chemicals

4-PSQ (Figure 1), was prepared and characterized in laboratory by the method previously described by Duarte et al. (2017). Sucrose PA ACS Fórmula: $C_{12}H_{22}O_{11}$ / molecular weight: 342.30. 1-ethanesulfonic acid (HEPES), 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), 7-Hydroxy-3H-phenoxazin-3-one 10-oxide (Resazurin), 2H-tetrazolium bromide (MTT), β−Mercaptoethanol, Ethylenediaminetetraacetic acid (EDTA), N,N,N′,N′-Tetramethylethylenediamine (TEMED), Quercetin, Sodium orthovanadate, thiobarbaturic acid (TBA) were obtained from Sigma-Aldrich. Triglycerides Liquiform and Glucose were obtained from Bioclin (Brazil). Tris(hydroxymethyl)aminomethane, hydrogen peroxide, dimethylsulfoxide (DMSO), TRITON X-100, and Dimethyl sulfoxide were purchased from Synth (Diadema, SP, Brazil).

2.2 Animals and diet

Drosophila melanogaster (Harwich strain) was obtained from the National Species Stock Center, Bowling Green, OH, USA. Flies in a proportion of 70% females and 30% males, from 1 to 5 days of age, were maintained for 10 days at 25 ° C in a light / dark cycle of 12 hours in glass vials containing 5 ml of diet (which consisted of 76.59% corn flour, 8.51% wheat germ, 7.23% sugar, 7.23% milk powder, 0.43% salt and nipagin).

2.3 Experimental Protocol

The composition of the regular diet (RD) and the hyperglycidic diet (HD) are described in Table 1. For the HD, the curve previously described by MORRIS et al., 2012 was used and the concentrations of 5%, 10% and 30% of sucrose as a variable element on the standard diet were defined. Sucrose was added directly into the diet. The compound was solubilized in ethanol and to define the concentration of 25 μM of the compound, the curve previously developed by COUTO et al. (2018) was used.

Flies were divided into 8 groups of 50 flies each, in the proportion of 70% females and 30% males, (1) RD, (2) RD + 4-PSQ 25 μM, (3) HD with 5% , (4) HD with 10% , (5) HD with 30% , (6) HD with 5% + 25 μM 4-PSQ, (7) HD with 10% + 25 μM of 4-PSQ, (8) HD with 30% + 25 μM of 4-PSQ (Figure 2).

2.4 Survival rates

The survival rate of the flies exposed to the treatments was evaluated daily, counting the number of live flies in relation to the number of dead flies at the end of the experimental period (10 days). Approximately 250 flies per group were included in the survival data and the total number of flies represents the sum of four independent experiments (50 flies per group).

2.5 Longevity and cumulative eclosion

To test the shelf-life, the flies after the 10-day treatment were kept on a standard diet, which was changed every two days until there were no more live flies. The results represent the mean of four independent experiments and are expressed as the number of flies per day.

The total hatching of treatment groups was obtained by counting the total number of flies born in each group (first day = 0 flies), after removal of the progenitors

from the medium, until there were no more viable eggs. The offspring were removed daily at the same time each day.

2.6 Preparation of homogenate

Twenty flies in a proportion of 70% female and 30% male, per group, were separated and immobilized by freezing on ice. Subsequently, the head and body were carefully separated and homogenized in HEPES buffer (20 mM, pH 7.0) according to each protocol analysis. After centrifugation, the supernatant was removed and used for the biochemical assays. All experiments were performed in duplicate.

2.7 Food intake

Groups of 15 headless flies were left for a fasting period of 12 hours and subsequently placed for 24 hours in 1g of the respective treatment diets, plus 5% FD & C Blue N^{o}. 1 (Brilliant Blue FCF). After the feeding period, each group of flies was immediately frozen and euthanized. The flies were then homogenized in 100 µL of 50 mM potassium phosphate buffer (KPI), pH 7.5, centrifuged at 13.500 rpm, for 15 min, and the supernatant transferred to a new tube. Samples were measured on a 96-well microplate reader at 629 nm. The optical density of the homogenates of the flies that consumed the corresponding diets without the dye was used as a blank. The final result was calculated in relation to the consumption of the flies of the control group. 60 flies were used for each treatment group and all experiments were performed in duplicate. Methodology adapted from LUSHCHAK et al. (2011) with adaptations.

2.8 Measurements of glucose, triglycerides activity

15 flies per group were anesthetized and euthanized. After removal of the heads were homogenized and prepared for the analysis of triglycerides as described by BIRSE (2010), for lipase homologs and glucose analyzes using the Bioclin® kit specific for each assay.

2.9 Protein

Protein concentrations of the homogenate were determined by the BRADFORD method (1976), using bovine serum albumin as standard. The results represent the mean of three independent experiments and are expressed in mg / dL.

2.10 Determination of thiobarbituric acid reactive substances (TBARS)

Fifteen flies in each group were homogenized in 300 μL of 20 mM HEPES buffer pH 7.0 and centrifuged at 1000 rpm for 10 min at 4°C. The supernatant was removed and thiobarbituric acid (0.8% TBA, pH 3.2), cloridric acid / HCl (20%, pH 3.5) and sodium dodecil sulfate (SDS 8.1%). The samples were then incubated for 2 h at 95ºC and the absorbance read at 532 nm. According to the methodology described by OHAKAWA., 1979. The results represent the average of four independent experiments (15 flies per group). TBARS values were normalized by protein concentration and expressed in nmol MDA / mg protein.

2.11 Determination of superoxide dismutase activity (SOD)

Ten flies from each group were homogenized in 100 uL HEPES buffer pH 7.0 and centrifuged at 14000 rpm for 30 min at 25ºC. The reaction mixture contained sodium phosphate buffer (0.025 M / 0.1 mM EDTA, pH 10) N,N,N',N'-Tetrametiletilendiamina (TEMED) and 10 μL of sample and was started by adding 0.15% of quercetin dissolved in dimethylformamide. At the time of reading the samples, quercetin was added and monitored for 2 min at 406 nm. SOD activity was measured by monitoring the inhibition of quercetin auto-oxidation, according to KOSTYUK and POTAPOVICH (1989), with modifications. The results were expressed in terms of the amount of protein required for 50% inhibition of quercetin oxidation. Four independent experiments were performed (10 flies per group). The enzymatic activity was expressed in U/mg protein.

2.12 Determination of catalase (CAT) activity

CAT activity was determined spectrophotometrically by the AEBI method, (1984) based on the ability of CAT to degrade H_2O_2, with some modifications. Kinetic analysis of CAT activity initiated from the addition of a potassium phosphate buffer solution (0.25 M / 2.5 mM EDTA, pH 7.0), 30% hydrogen peroxide (H_2O_2) and Triton X- 100. The decrease in optical density at 240 nm was measured for 2 min at 25°C and the results were linear in relation to time and amount of sample. Activity was expressed as units of CAT per mg protein (U/ mg protein).

2.13 Determination of thiol levels

Thirty-five flies from each group without the head, were homogenized with subsequent addition of 0.5 M perchloric acid and the samples were centrifuged at 10.000 rpm for 5 min at 4°C. For non protein thiol (NPSH) measurements, the supernatant was used and 5 mM DTNB. For protein thiol (PSH) samples, the pellet was resuspended in 0.5 M Tris / HCl (pH 8.0), the supernatant was removed and 5 mM DTNB added, the samples were kept for 15 minutes at room temperature protected from light and the reading was performed by spectrophotometry at 412 nm. Four independent experiments (35 flies per group) were performed. Results were expressed as a percentage (%) of the control group.

2.14 Resazurin reduction test cell

The resazurin reduction test was performed with 10 separate head and body flies from each treatment. The flies were anesthetized on ice and homogenized. The procedure was performed with the CellTiter-Blue® cell viability assay kit, according to FRANCO (2009). The method relies on the ability of viable cells to reduce resazurin to resorufin, a fluorescent molecule. The flies were homogenized in 10 mM Tris buffer, pH 7.4 and 100 µL pipetted into a 96-well plate and an aliquot of CellTiter-Blue was added according to the manufacturer's instructions. After 1 h, the absorbance was recorded at 573 nm. The results represent the mean of three independent experiments and are expressed as a percentage of the control group.

2.15 MTT Reduction Test

The method of analysis was as described by HOSAMANI (2013). Briefly, the flies were placed in the 96-well plate with separate head and body, then 200 uL of [4,5-dimethylthiazol-2-yl 3-bromide]-2,5-diphenyltetrazolium MTT (Ul solution) for 1 hour at 37°C. Thereafter the MTT was removed and 200 microliters of dimethylsulfoxide (DMSO) added after incubation for 30 min at 37°C, absorbance was measured at 540 nm. The results were expressed as a percentage of the control.

2.16 Statistical Analysis

Differences in lifespan were analyzed by comparing the survival in eclosion curves by log-rank (Mantel-Cox) test. Data were analyzed using Prism 6 software (GraphPad). The comparisons between the experimental and control groups were performed by univariate analysis of variance (ANOVA), followed by Newman Keuls

post hoc or two-way ANOVA, followed by the Bonferroni post hoc test, when appropriate. Descriptive statistics were expressed as the mean (s) ± S.D. Values of probability less than 0.05 ($P < 0.05$) were considered statistically significant.

3. RESULTS

3.1. 4-PSQ increases the survival rate and longevity of *Drosophila melanogaster*

According to Figure 3A, co-treatment of adult flies to HD at concentrations of 5%, 10% and 30% caused an increase in mortality in the experimental period of 10 days when compared to RD. Co-treatment with 4-PSQ reduced mortality and increased the total life of flies. The longevity of the groups receiving HD of 30% was on average up to 36 days, however, the flies co-treated with 4-PSQ increased the time life by more than 33%, causing the fruit flies to live in to 54 days (Figure 3B). These data show the ability 4-PSQ compound to protect against mortality and prolong life the *Drosophila melanogaster* when exposed to HD ($p < 0.05$).

3.2 The 4-PSQ causes an increase in the rate of hatched eggs in *Drosophila melanogaster*.

The HD diet reduced the number of viable egg hatchings of Drosophila melanogaster at all concentrations (5%, 10%, and 30%, respectively), 4-PSQ increased hatching mainly in the first 10 days (Figure 4A). According to Figure 4B, HD at all concentrations promotes a significant decrease in the cumulative eclosion rate of flies compared to the RD group, the reduction of viable egg hatching was efficiently reversed by the 4-PSQ compound ($F = 8.021$, $P = <0.0003$; One way ANOVA).

3.3 Fruit flies co-treated with 4-PSQ had a change in the pattern of food consumption

Flies exposed to the HD diet 10% and 30% consumed more food than the RD group (Figure 5). The co-treatment with 4-PSQ showed a satiety effect in the flies that presented lower food consumption when compared to the HD groups. ($F = 4.127$, $P = <0.0045$; One way ANOVA).

3.4 4-PSQ reduces glucose levels and triglycerides levels.

According to Figure 6A, there was an increase in glucose levels in all HD concentrations (2.5, 2.86 and 4.04 times respectively). The Co-treatment with compound 4-PSQ was efficient in reducing levels only in HD 30% (F = 16.09, P = <0.0001). The triglyceride levels observed in the body of the flies from 10% and 30% HD were, respectively, 3.08 and 2.13 times higher than the values found in the RD group. The 4-PSQ compound restored triglyceride levels in the two largest concentrations de HD (Figure 6B) (F = 9.499, P = <0.0001; One Way ANOVA).

3.5 4-PSQ improves the parameters of oxidative stress in flies fruit exposed to an HD.

Figure 7A shows a 1.6-fold increase in TBARS formation, in HD 30%, which is an indicator of lipid peroxidation, when compared to the RD group. The 4-PSQ was effective in reversing this increase (F = 9.453, P = <0.0001; One way ANOVA). The activity of the antioxidant enzymes SOD and CAT was increased in flies fed with HD. In SOD the HD intake at all concentrations (represented by the inhibition of quercetin oxidation), caused an increase of 4.67, 4.49 and 3.91 times in enzyme activity when compared to the values found in the RD group (Figure 7B) . Compound 4-PSQ was efficient in normalizing activity, reducing the values in all HD concentrations (F = 9.859, P = <0.0001; One Way ANOVA). Differently from SOD, there was an increase in CAT activity when compared to the RD group only at the highest HD concentration (30%); however, two concentrations of the diet receiving 4-PSQ showed a reduction in activity when compared to their respective controls (F= 9.661, P=< 0.0001; One Way ANOVA) (Figure 7C).

3.6 Determination of the levels of protein in non- protein thiols

In Figure 8A there was no significant difference in the levels of non-protein thiols (NPSH) between the HD and 4-PSQ diet groups (F = 0.2874, p <0.05; One Way ANOVA). Although, in protein thiols (PSH) there was an increase in the HD 10% + 4-PSQ group in relation to their respective control (Figure 8B), (F = 0.2874, P = 0.0105, One Way ANOVA).

3.7 In cell viability assays

The resazurin reduction test showed no significant difference at any of the concentrations of the HD (F= 1.394, P=< 0.2531; One Way ANOVA), (Figure 9A). In

addition there was a significant increase in MTT reduction in the fraction of cells in the HD 5% group when compared to the control group and a reduction in the levels of co-exposed groups containing HD 5% and 10% + 4PSQ with compared in respective groups control (Figure 9B) (F= 74.57, P= <0.0001; One Way ANOVA).

4. DISCUSSION

In general high consumption of hyperglycidic diets negatively affected the survival and shelf life of *Drosophila*. *melanogaster* in our study. These effects were accompanied by elevation of glucose, triglycerides and TBARS levels and increase on lipase, SOD and CAT activities, as well as reduction of viable egg hatching. Several studies using *Drosophila melanogaster* emphasize the relationship between consumption of a hyperglycidic diet with metabolic alterations (ECKER et al., 2017; MUSSELMAN and KÜHNLEIN 2018; ALFA and KIM 2016).Our results corroborate the study by Ecker et al., 2017 that when exposed to diets rich in sucrose found reduced larval development and decreased survival rate of adults.

The present study revealed for the first time that 4-PSQ, a quinoline derivative with an organoselenium substituent, has a protective effect on the metabolism of *Drosophila melanogaster* in a diabetes-like model induced by hyperglycidic diet, preventing oxidative stress and restoring antioxidant defenses. The 4-PSQ compound reduced flies mortality during the 10 days of treatment and this effect lasted over the entire life of the flies. Peculiarly, the group with the highest survival was the group that received 30% sucrose + 4-PSQ, while the group with the lowest longevity was the group that received the 30% sucrose diet.

Longevity may also be associated with insulin signalling. In the study by HOFFMAN et al., (2014) the experimental models that presented higher life expectancy, frequently present alterations in the insulin pathway, the authors carried out studies in long-lived *Drosophila* strains and found that metabolites that decrease with age are associated glycolysis and glycoprotein metabolism. The mechanisms that lead to greater survival in *Drosophila melanogaster* have not yet been fully elucidated, but possibly the effect of the 4-PSQ compound acts on different lines of antioxidant defenses, and evidence in the literature indicates that 4-PSQ has a ferric ions reducing capacity, which is an indicator (VOGT et al., 2018), because an important aspect to be highlighted is the existence of an imbalance in the antioxidant defense system in the

presence of type 2 diabetes. In this sense, the 4-PSQ selenium/quinoline has been shown to be an compound with relevant function in the antioxidant defense system.

It is known that dietary intake is the product of meal size and meal frequency, it has been shown that selenium compounds have possible actions to increase satiety in rats (BORTOLATO et al., 2015). Our study demonstrates for the first time that the effect of increased satiety and alteration in the dietary pattern can be obtained with 4-PSQ in *Drosophila melanogaster*. The results demonstrate that the flies that consumed the hyperglycidic diets associated with 4-PSQ reduced the frequency of meals and the total volume of food consumed, an effect opposite those who consumed only the hyperglycidic diets. The anorexic effect can be observed in the two highest concentrations of sucrose 10% and 30%, and corroborate with BORTOLATTO et al., 2015, which demonstrated that rats treated with selenium compounds diselenides $(PhSe)_2$ presented a satiation process facilitation.

In mammals, food intake is regulated at the hypothalamic level by orexigenic and anorectic neuropeptides that are under the control of peripheral signals such as leptin. Increased leptin levels and decreased ghrelin levels may translate into a situation of resistance to leptin and hypersensitivity to ghrelin, such changes impair the regulation of satiety (ANTONOVA et al. 2012; GRÖNKE et al 2010). In the *Drosophila melanogaster* fly there are neurosecretory cells in the brain that secrete insulin, as well as additional secretory cells that secrete a glucagon analogue that together exhibits physiological and genetic parallels to the endocrine axis of vertebrates (PANDAY and NICHOLS, 2011) In excess, carbohydrate consumption promote the inharmony of metabolic homeostasis, triggering a process of continuous and uncontrolled inflammation, which is the main damaging factor of DM2, since the characteristic permanent inflammatory process has a high causal effect on adipokines, which interfere throughout the body. The 4-PSQ compound possibly acts regulating appetite at the center of hunger or potentiates the mechanisms related to food intake, co exposure of 4-PSQ in the appetite and food intake pathways need to be better elucidated in *Drosophila melanogaster*.

Metabolic changes, such as enzymatic dysregulation and elevated levels of glucose and triglycerides, are an important pathophysiological mechanism closely related to glucose intolerance and DM2 in mammals (ECKEL et al., 2011; SAMUEL and SHULMAN, 2012). Our study demonstrated that the hyperglycidic diet, in concentrations 10% and 30%, increased glucose and triglyceride levels in *Drosophila*

melanogaster. In the fed state, circulating carbohydrates are abundant and anabolic actions of insulin predominate, including the uptake of glucose by the liver in mammals (and by the fatty body in *Drosophila*), as well as glycogen synthesis and lipolysis decrease (SAMUEL and SHULMAN, 2012). Under conditions of insulin resistance, peripheral tissues do not respond to insulin, resulting in hyperglycemia, dysregulated glycogen synthesis and elevation of circulating free fatty acids of inadequate lipolysis (SAMUEL and SHULMAN, 2012), the results reinforce the diabetic phenotype and the flies exposited with 4PSQ obtained an improvement in glucose and triglyceride levels.

The antioxidant systems of the cells are altered in DM2, causing an imbalance in their redox state. The data presented in our study show that there is an increase in lipid peroxidation (TBARS) at the concentration of 30% sucrose and this increase is reversed by the compound 4-PSQ demonstrating that the 4-PSQ is a multi-target molecule, protecting against increased levels of TBARS in the body of the fly. The relationship between diabetes, especially type 2 and oxidative stress has been widely studied. Oxidative stress is an imbalance between oxygen/nitrogen, free radical production and endogenous physiological antioxidant mechanisms (ROBSON et al., 2018). The compromise of physiological antioxidant defenses may be a cause of increased oxidative stress in individuals with DM2. According to the literature, the pathogenesis of DM2 is multifactorial and therefore it is crucial to identify the specific molecules related to oxidative stress and its associated production pathways in order to develop an effective and targeted therapy (FORMAN et al., 2014).

4-PSQ attenuated the deleterious effects of the hyperglycidic diet on biomarkers of oxidative stress, as expected there was an increase in TBARS values in HD 30%, possibly due accumulation to lipid oxidation, as well as nucleic acids, proteins and sugars, with co-exposure with 4-PSQ levels were normalized in relation to the control group. It is to be expected that doses of the selenium-containing compound have been able to reduce the cascade reaction of free radical formation. These findings clearly demonstrate that the compound plays a defensive role against oxidative stress damage in *Drosophila melanogaster*.

In diabetic individuals, low levels of antioxidant enzymes or excess free radical production lead to the removal of defective reactive oxygen species causing deregulation of the enzymatic activity (PALM et al., 2012). According to the results presented herein, the HD diet causes changes in the activities of SOD and CAT enzymes. Our results corroborate with ECKER et al., 2017 who observed a significant

change in the activities of SOD and CAT enzymes in flies exposed to the hyperglycidic diet. In this study the activities of these enzymes were reestablished in the co-exposed groups with 4-PSQ, a trend similar to TBARS. These results suggest that 4-PSQ induces a protective effect on the *Drosophila melanogaster* organism exposed to the HD diet, aiding the elimination of reactive oxygen species, thus reducing the complications associated with diabetes.

Our study demonstrated for the first time the effect of 4-PSQ compound on lipase activity in flies exposed to HD diet. Lipases are hydrolytic enzymes that decompose triglycerides into free fatty acids and glycerol and operate on the surface of emulsified lipid substrates (PISTILLO et al., 1998). The biochemical and molecular characteristics shown by lipases indicate that they are involved in a wide variety of reactions and are products of gene families that differ in their amino acid sequence, tissue expression specificity, and cell compartmentalization. In *Drosophila melanogaster* there is the identification and characterization of three genes whose translation products show a high level of similarity with mammalian lipases.

The elevation of the activity of lipase enzymes is a challenge, and there are few studies that clarify its meaning. It has been proposed that insects produce lipase homologues exclusively in the fat body, which functions in a manner analogous to the mammalian liver and adipose tissue (PALM, 2012). Possibly the disturbed lipid metabolism affects the lipid composition in individual organs, this may reflect a dominance of the nutritional lipid uptake resulting in imbalance of the enzymatic levels.

5. CONCLUSION

Our results suggest that 4-PSQ has a multifactorial action in the metabolism of *Drosophila melanogaster,* as satiety effect and improvement of the antioxidant function, acting in the decrease of glucose and triglyceride levels, in the formation of reactive species and in the reversion of the increase in SOD activity, CAT caused by consumption of hyperglycidic diet. The study shows the pharmacological potential of this quinoline derivative containing selenium for the treatment of metabolic disorders, similar to diabetes. Therefore, further studies on the subject are needed to elucidate the mechanisms of action of 4PSQ on the manifestation of type 2 diabetes mellitus and the disorders associated with it.

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Figure Captions

Figure 1. chemical structure of the compound 7-chloro-4- (phenylselanyl) quinoline (4-PSQ).

Figure 2. Experimental protocol.

Figure 3. 4-PSQ improves the survival rate in *D. melanogaster*. A) 10-day survival curve: the number of live and dead flies was counted every 24 hours for 10 days of treatment and dietary exposure. B) Shelf life: flies were counted daily until there were no more live flies. Mortality of flies exposed to HD associated with 4-PSQ (a, b and c) significantly reduced fly mortality compared to flies exposed only to hyperglycidic diets ($p < 0.05$, two-way ANOVA). Three groups of 50 flies were used for each dietary condition (mean ± standard deviation), $p < 0.05$ compared to the control group. (*) P value summary.

Figure 4. HD causes reduction of hatching rate in *D. melanogaster*. After 10 days of exposure, the progenitor flies of the respective treatments were removed and the eggs were monitored daily until hatching. (4A) the data show the hatching of eggs per day (1st to 17th). (4B) The data express the accumulated hatching until there were no more viable eggs * $P < 0.05$ in relation to RD, (a, b, c) 5%, 10% and 30% sucrose groups that received 4-PSQ increased the rate of hatched eggs, in relation to the groups that consumed only the diets.

Figure 5. Amount of food consumed by D. melanogaster in RD, HD (5%, 10%, and 30%) and HD + 4-PSQ. Data are presented as means ± S.E.M. In each test group, 15 flies were used in 4 replicates. * total intake different from the control group, (a, b) groups HD 10% and 30% who received 4-PSQ had reduced intake, in relation to the groups' RD $P < 0.05$.

Figure 6. HD increased glucose levels (6 A) triglycerides and (6 B) in *D. melanogaster*. Four sets of twenty flies from each treatment were used after 10 days, the results were

expressed as mean (mg / dL) ± standard deviation. * p <0.05 in relation to the control group, (a, b, c) difference of 4-PSQ in relation to the respective diet-only group.

Figure 7. 4 PSQ improves parameters of oxidative stress in flies exposed to hyperglycidic diet. Flies exposed to both diets were homogenized and the supernatant was used for stress marker analyzes and the activity of antioxidant enzymes. (7A) shows the end products of lipid peroxidation determined by the TBARS assay on the total flue homogenate, expressed on average (nmol MDA / mg tissue) ± standard deviation. (7B) shows the activity of superoxide dismutase (SOD) expressed as the average (amount of protein required to inhibit 50% automatic quercetin oxidation in U/mg protein) ± standard deviation in the total homogenate of flies and (7C) shows the activity Catalase (CAT) expressed as mean (U/mg protein H_2O_2 decomposed /min/mg protein) Five sets of 20 flies were analyzed for each dietary condition. * Indicates a significant difference in relation to the control group (P <0.05), a, b, c indicates groups containing 4-PSQ different from their respective controls.

Figure 8. Effect of HD on the content (A) of non-protein thiols and (B) on the homogenate content of protein thiols. The results were expressed as the percentage (%) of the control group (mean ± standard deviation and p <0.05). (n = 35 flies per replicate and four treatments were performed). Significance was determined by the analysis of two-way variance (ANOVA) followed by the Bonferroni test * Significant difference compared to the control group; the significant difference between the 10% sucrose groups containing 4-PSQ in relation to their respective control group (p <0.05).

Figure 9. Effects of 4-PSQ on metabolic activity and dehydrogenases on the total homogenate of flies treated for 10 days with standard diet and 4-PSQ. For the resazurin reduction test (9A) (three sets of 20 flies were analyzed for each diet condition), for the MTT (9B) assay approximately 60 mg of fly tissue were analyzed for each diet. Both graphs express the results as the percentage (%) in relation to the RD group (mean ± standard deviation). * (P <0.05) in relation to the control group. a, b expression difference between the 5% and 10% groups containing 4-PSQ with their respective controls.

Table 1

Tab 01 composition of regular diet (RD) and hiperglycid diet (HD)%

	RD	HD 5%	HD 10%	HD 30%
Carbohydrate (weight, %)	75,2	76,53	78,56	89,88
Protein (weight, %)	9,07	8,92	7,82	6,81
Total fat (weight, %)	2,29	2,26	1,90	1,76
Energy (kcal/g)	4,19	4,43	5,02	6,81

5%, 10% and 30% HD: hyperglycidic diets with sucrose addition.

Figures

Figure 1.

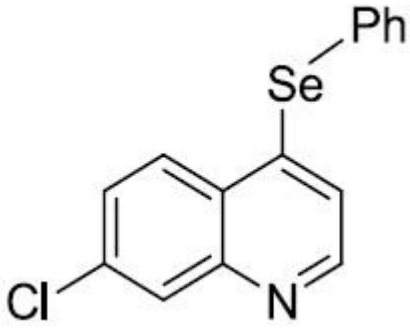


Figure 2

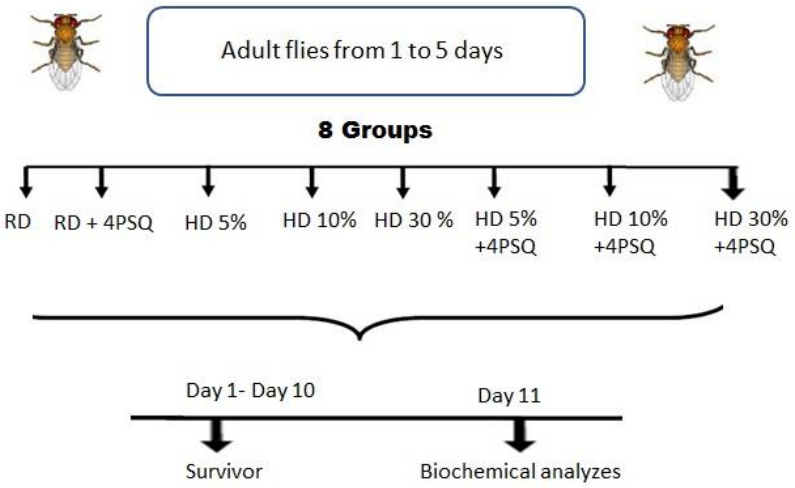
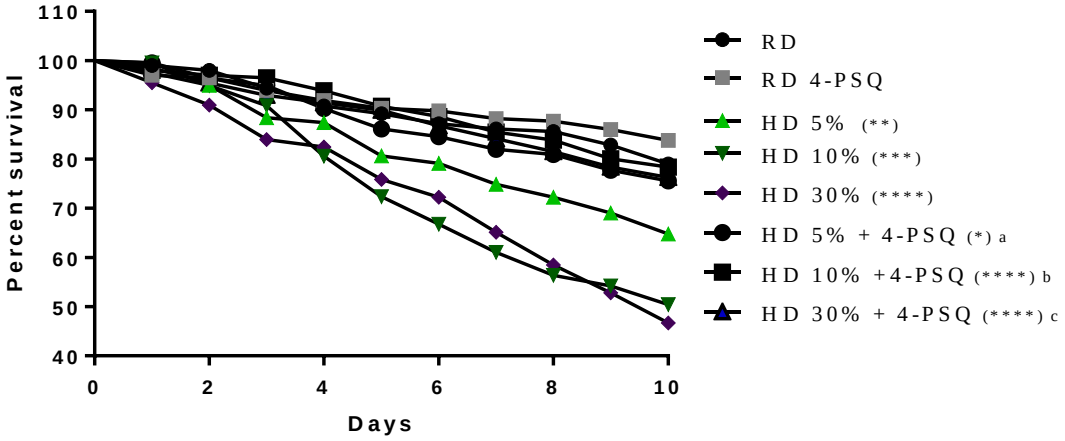
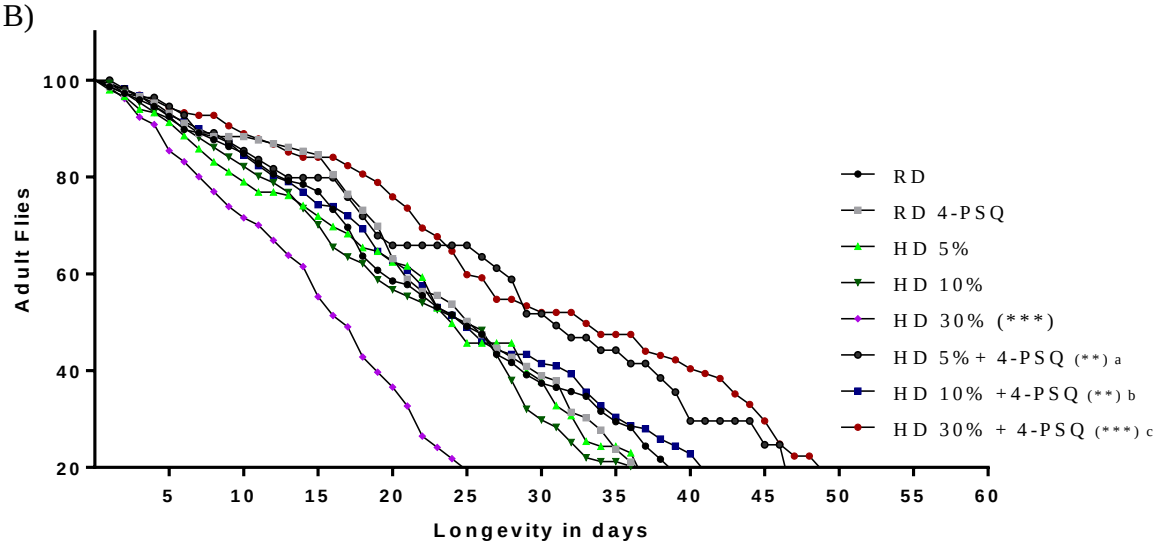


Figure 3 A)



B)


Adult Flies
100
80
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40
20
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25
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45
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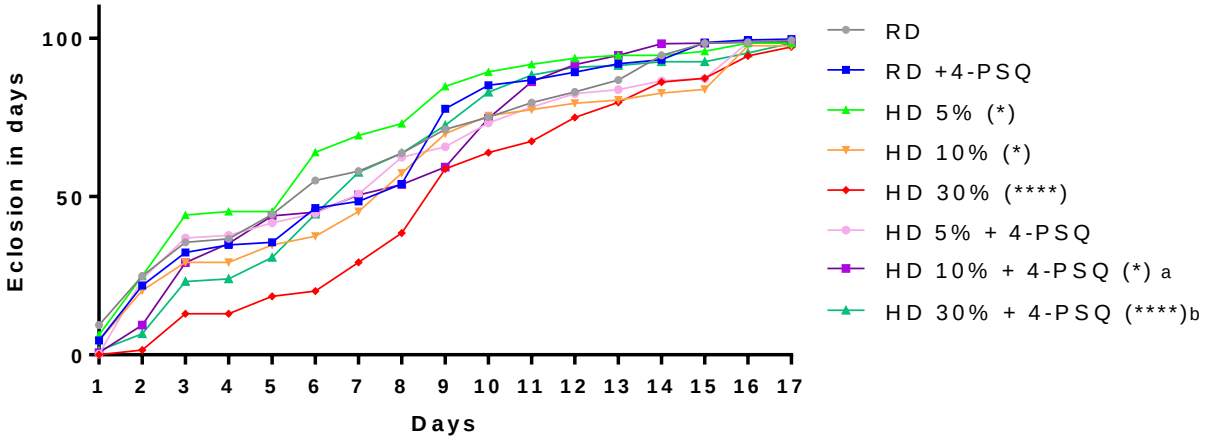
55
60
Longevity in days
RD
RD 4-PSQ
HD 5%
HD 10%
HD 30% (***)
HD 5% + 4-PSQ (**) a
HD 10% +4-PSQ (**) b
HD 30% + 4-PSQ (***) c

Figure 4

A)


Eclosion in days
100
50
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
Days
RD
RD +4-PSQ
HD 5% (*)
HD 10% (*)
HD 30% (****)
HD 5% + 4-PSQ
HD 10% + 4-PSQ (*) a
HD 30% + 4-PSQ (****)b

B)

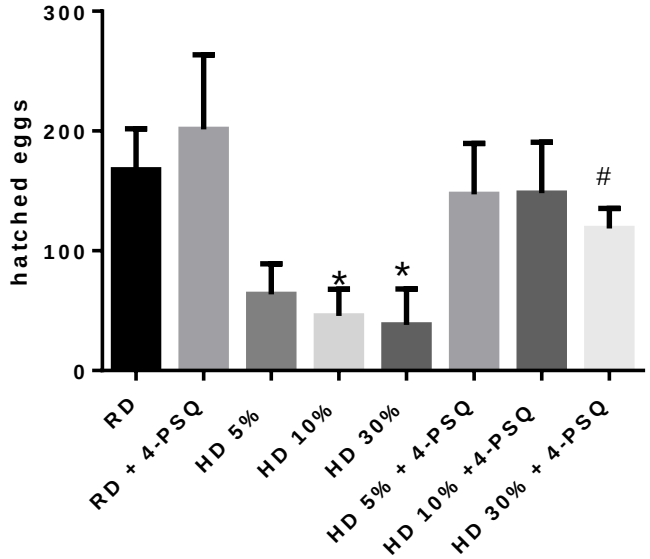

hatched eggs
300
200
100
0
*
*
#
RD
RD + 4-PSQ
HD 5%
HD 10%
HD 30%
HD 5% + 4-PSQ
HD 10% +4-PSQ
HD 30% + 4-PSQ

Figure 5

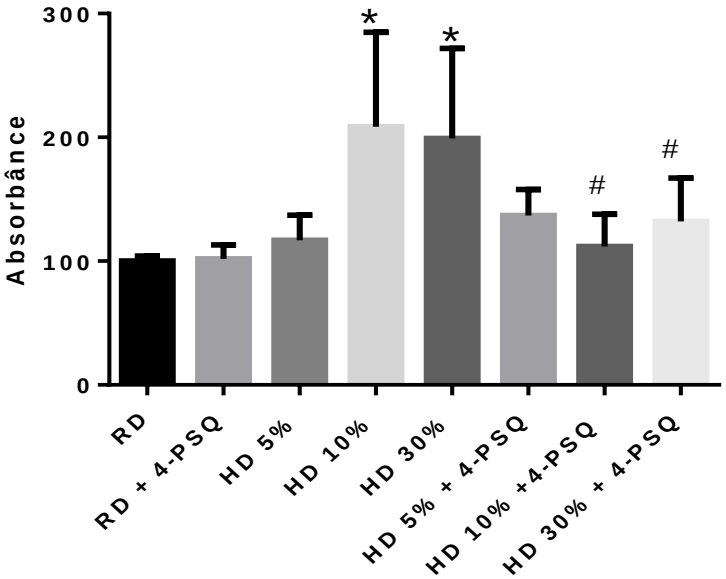
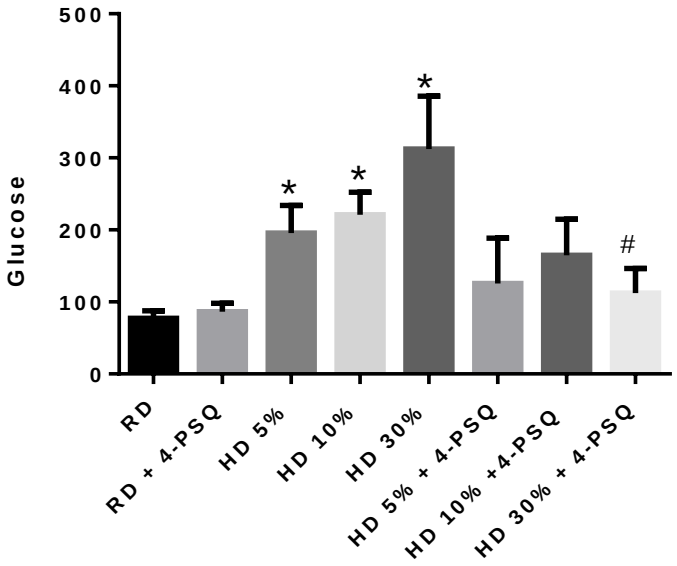


Figure 6

A)



B)

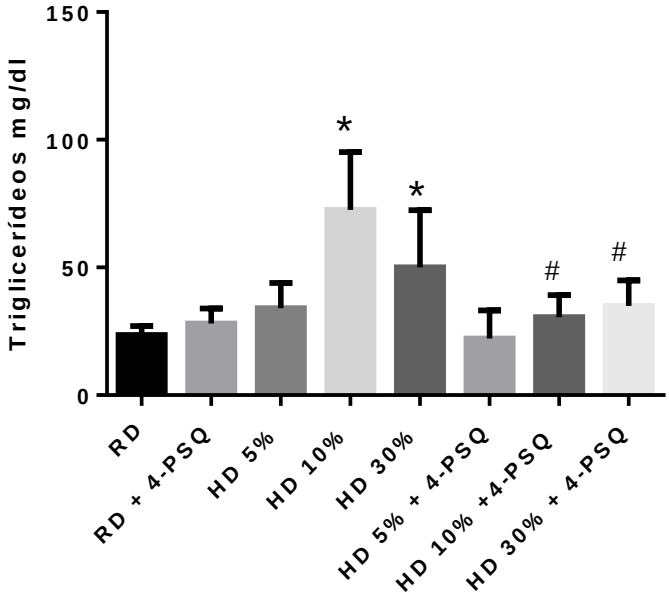
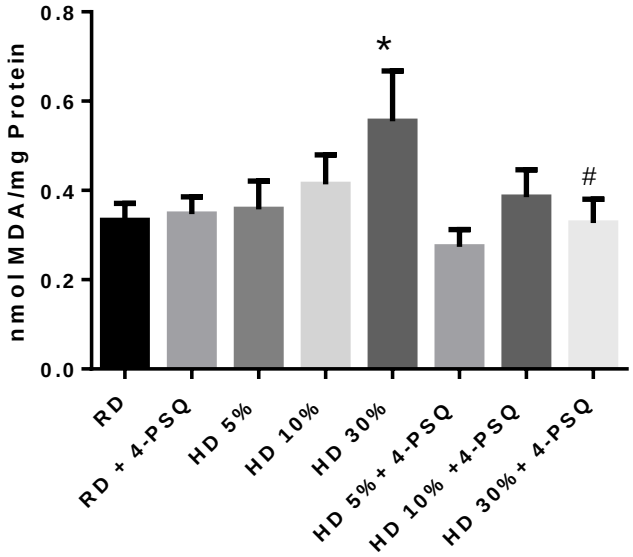
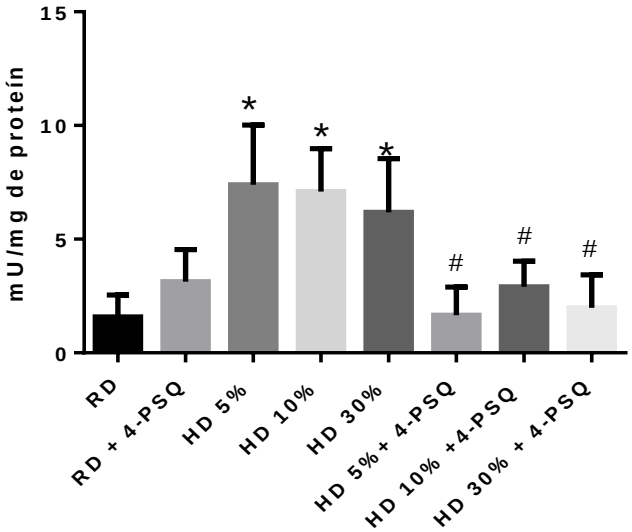


Figure 7

A)


nmol MDA/mg Protein
0.0
0.2
0.4
0.6
0.8
*
#
RD
RD + 4-PSQ
HD 5%
HD 10%
HD 30%
HD 5%+ 4-PSQ
HD 10% +4-PSQ
HD 30%+ 4-PSQ

B)


mU/mg de proteín
0
5
10
15
*
*
*
#
#
#
RD
RD + 4-PSQ
HD 5%
HD 10%
HD 30%
HD 5%+ 4-PSQ
HD 10% +4-PSQ
HD 30% + 4-PSQ

C)

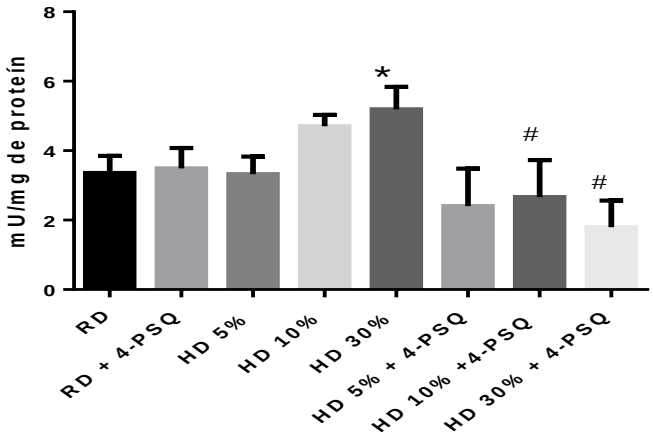

mU/mg de proteín
0
2
4
6
8
*
#
#
RD
RD + 4-PSQ
HD 5%
HD 10%
HD 30%
HD 5% + 4-PSQ
HD 10% +4-PSQ
HD 30% + 4-PSQ

Figure 8

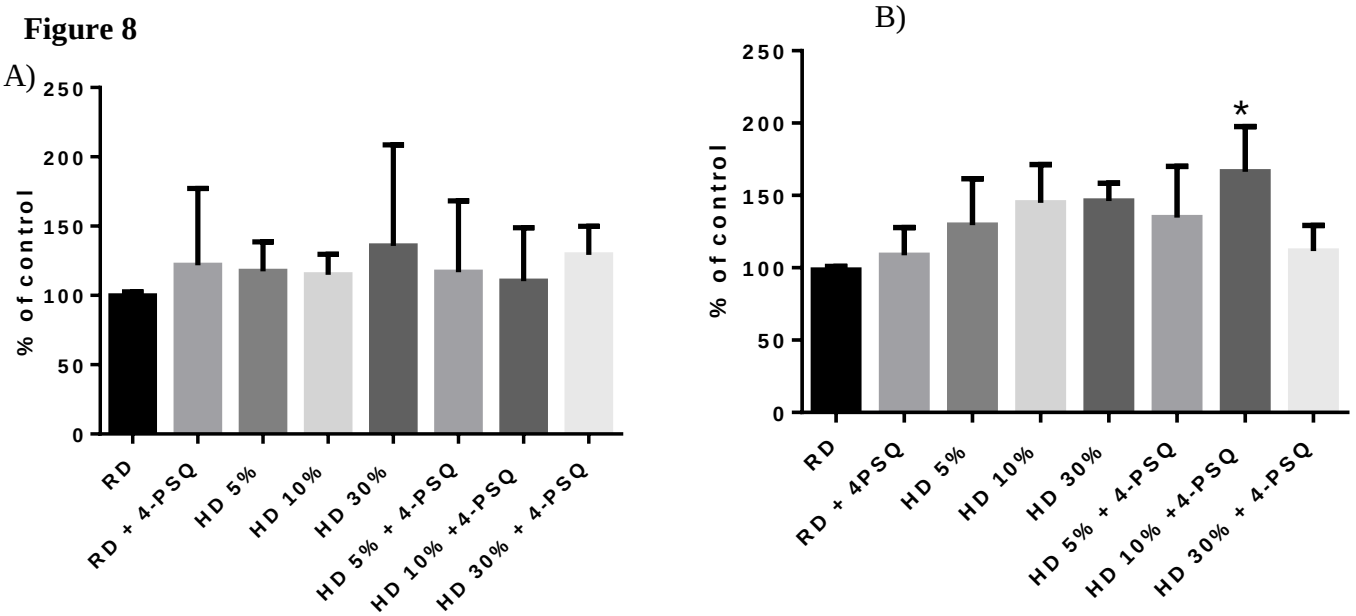
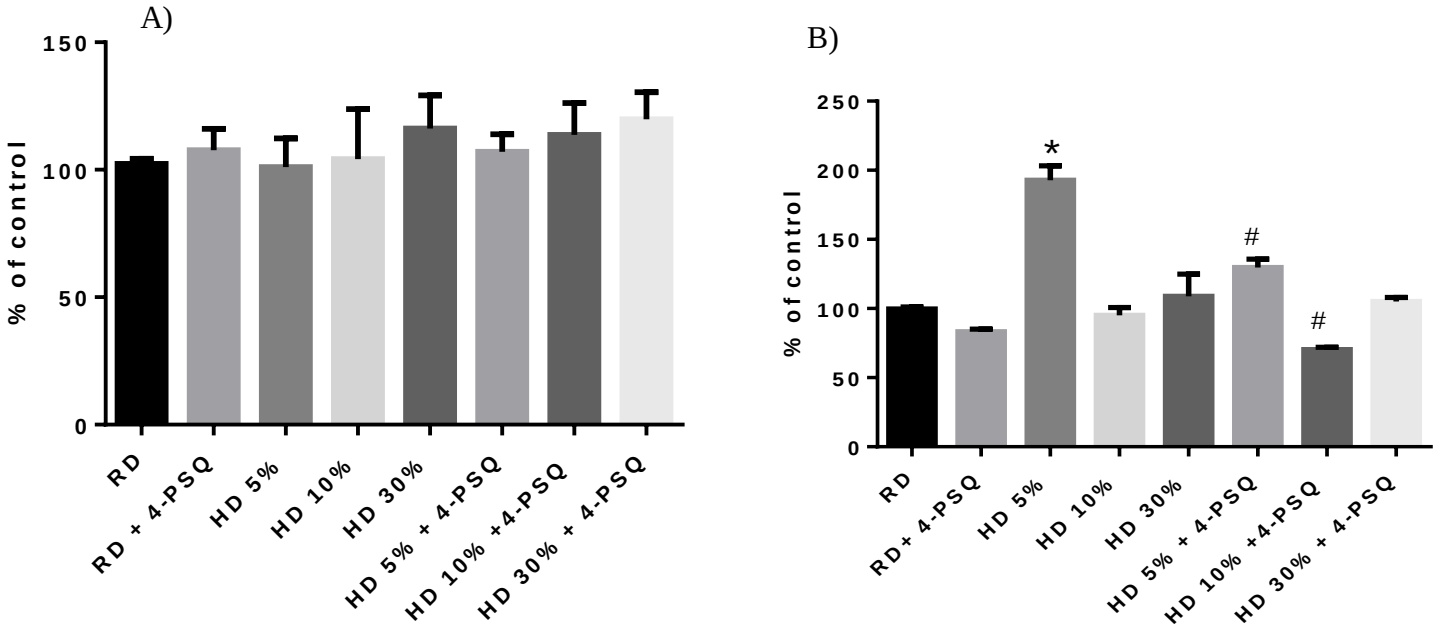


Figure 9



CONCLUSÃO

♦ O composto 4-PSQ aumentou a taxa de sobrevivência e longevidade de *D. melanogaster* após exposição à dieta hiperglicídica.

♦ O tratamento com 4-PSQ reduziu os níveis de glicose e triglicerídeos em *D. melanogaster* após exposição crônica à dieta hiperglicídica.

♦ O tratamento com 4-PSQ aumentou a taxa de eclosão e reduziu o percentual de ingestão alimentar de *D. melanogaster* após exposição crônica á dieta hiperglicídica.

♦ O composto 4-PSQ reduziu o estresse oxidativo após a exposição crônica à dieta hiperglicídica em *Drosophila melanogaster*.

♦ O tratamento com composto 4-PSQ não alterou a viabilidade celular e mitocondrial de *D. melanogaster* após exposição crônica a dieta hiperglicídica.

PERSPECTIVAS FUTURAS

Baseado nos resultados adquiridos, obtemos o interesse em ampliar o conhecimento a respeito do efeito do 4-PSQ no modelo de diabetes, elucidando quais mecanismos estão envolvidos no efeito deste composto, assim como verificar seu efeito a longo prazo e em descentes dos indivíduos expostos ao tratamento.

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