UNIVERSIDADE FEDERAL DO PAMPA

FRANCIELLI POLET DE ALMEIDA

7-CLORO-4-(FENILSELANIL) QUINOLINA PROTEGE CONTRA ALTERAÇÕES COMPORTAMENTAIS, OXIDATIVAS E COLINÉRGICAS INDUZIDAS PELA EXPOSIÇÃO À ACRILAMIDA EM *Drosophila melanogaster*

Uruguaiana

2021

FRANCIELLI POLET DE ALMEIDA

7-CLORO-4-(FENILSELANIL) QUINOLINA PROTEGE CONTRA ALTERAÇÕES COMPORTAMENTAIS, OXIDATIVAS E COLINÉRGICAS INDUZIDAS PELA EXPOSIÇÃO À ACRILAMIDA EM *Drosophila melanogaster*

Dissertação apresentada ao programa de pós-graduação *Stricto Sensu* em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do grau de Mestre em Bioquímica.

Orientadora: Prof.ª Dra. Marina Prigol

Coorientador: Prof. Dr. Gustavo Guerra

Uruguaiana

2021

Ficha catalográfica elaborada automaticamente com os dados fornecidos
pelo(a) autor(a) através do Módulo de Biblioteca do
Sistema GURI (Gestão Unificada de Recursos Institucionais) .

A4477 Almeida, Francielli Polet de
7-cloro-4-(fenilselanil) quinolina protege contra alterações comportamentais, oxidativas e colinérgicas induzidas pela exposição à acrilamida em Drosophila melanogaster / Francielli Polet de Almeida.
65 p.

Dissertação(Mestrado)-- Universidade Federal do Pampa, MESTRADO EM BIOQUÍMICA, 2021.
"Orientação: Marina Prigol".

1. Antioxidante. 2. Anticolinesterásico. 3. Compostos de selênio. 4. Quinolinas. I. Título.

FRANCIELLI POLET DE ALMEIDA

7-CLORO-4-(FENILSELANIL) QUINOLINA PROTEGE CONTRA ALTERAÇÕES COMPORTAMENTAIS, OXIDATIVAS E COLINÉRGICAS INDUZIDAS PELA EXPOSIÇÃO À ACRILAMIDA EM *Drosophila melanogaster*

Dissertação apresentada ao programa de pós-graduação *Stricto Sensu* em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do grau de Mestre em Bioquímica.

Área de concentração: Bioprospecção Molecular

Dissertação defendida e aprovada em: 24 de agosto de 2021.

Banca examinadora

_____

Prof.ª Dr.ª Marina Prigol

Orientadora (UNIPAMPA)

_____

Prof.ª Dr.ª Cristiane Luchese

(UFPEL)

_____

Prof. Dr. Carlos Borges Filho

(UNIPAMPA)

Assinado eletronicamente por **MARINA PRIGOL**, **PROFESSOR DO MAGISTERIO SUPERIOR**, em 23/09/2021, às 19:24, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.

Assinado eletronicamente por **Cristiane Luchese**, **Usuário Externo**, em 23/09/2021, às 19:53, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.

Assinado eletronicamente por **CARLOS BORGES FILHO**, **Técnico de Laboratório Área**, em 23/09/2021, às 21:33, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.

A autenticidade deste documento pode ser conferida no site https://sei.unipampa.edu.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=0, informando o código verificador **0622328** e o código CRC **1F450C09**.

AGRADECIMENTOS

Agradeço à Universidade Federal do Pampa por todas as oportunidades concedidas desde a graduação.

Agradeço ao Programa de Pós-Graduação em Bioquímica da Unipampa e ao seu corpo docente, por todo o conhecimento transmitido, que foi fundamental para que esta etapa fosse concluída.

Agradeço aos funcionários da Universidade Federal do Pampa, que contribuíram direta e indiretamente para a conclusão deste trabalho.

Agradeço à Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pelo apoio financeiro concedido em forma de bolsa de estudos durante o meu mestrado, sem ele, este trabalho não teria sido possível.

Agradeço ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e ao Fundo de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) por todo o suporte financeiro concedidos aos demais colegas e projetos do nosso grupo de laboratório.

Agradeço aos meus pais, por me apoiarem, por investirem em mim e por tornarem essa jornada possível.

Agradeço à minha orientadora Dr.ª Marina Prigol, pelas valiosas sugestões neste trabalho, obrigada pelo conhecimento científico, pelo incentivo, motivação para enfrentar os desafios e por todos os seus ensinamentos.

Agradeço ao meu coorientador, Dr. Gustavo Guerra, por me permitir participar da rotina do seu grupo de pesquisa, com o qual muito aprendi e pela contribuição prestada nesta dissertação.

Agradeço à Dr.ª Márcia Rósula Poetini, pela amizade, carinho, paciência, dedicação em todos os momentos desta caminhada.

Agradeço à Elize Musachio, pelo seu apoio e prontidão em auxiliar neste projeto.

Agradeço aos colegas do Laftambio pela convivência, amizade e troca de conhecimentos.

Agradeço aos membros da banca examinadora, Dr.ª Cristiane Luchese e Dr. Carlos Borges Filho, pelo interesse, disponibilidade, ideias, sugestões, críticas e considerações acerca desta dissertação.

RESUMO

A acrilamida (ACR) é um contaminante formado quando alimentos ricos em carboidratos são aquecidos em altas temperaturas (acima de 120°C) e sua formação ocorre através da reação de Maillard. Diversos estudos relataram que a ACR pode induzir genotoxicidade, carcinogenicidade e neurotoxicidade. É sugerido que o estresse oxidativo e alterações na atividade da enzima acetilcolinesterase (AChE) possam estar envolvidas na neurotoxicidade causada pela ACR, desta forma, compostos com propriedades antioxidantes e anticolinesterásicas como 7-cloro-4-(fenilselanil)quinolina (4-PSQ) poderiam prevenir ou retardar a neurotoxicidade causada pela ACR. Sendo assim, o objetivo deste estudo foi avaliar se o 4-PSQ, um composto orgânico de selênio, possui efeito protetor contra a indução de dano causado pela ACR em *Drosophila melanogaster*. Moscas adultas (ambos os sexos), entre 1 a 3 dias de idade foram divididas em 4 grupos de 50 moscas cada: Controle; 4-PSQ (25µM); ACR (5mM) e ACR (5 mM) + 4-PSQ (25µM) +. As moscas foram expostas à dieta durante 4 dias de acordo com seus respectivos grupos. As moscas expostas à ACR tiveram redução na taxa de sobrevivência, atividade locomotora e comportamento exploratório e aumento na atividade da acetilcolinesterase (AChE). A neurotoxicidade também foi associada a um aumento acentuado nas espécies reativas (RS), aumento nos níveis de malondialdeído (MDA), diminuição na atividade da superóxido dismutase (SOD), aumento na atividade da catalase (CAT), aumento na atividade da glutationa S-transferase (GST) e diminuição da viabilidade celular na cabeça das moscas comparadas ao grupo controle. Este estudo revela, pela primeira vez, que o co-tratamento com 4-PSQ em *Drosophila melanogaster* diminuiu a mortalidade e protegeu contra déficits locomotores, atividade da AChE, níveis de espécies reativas (ERs) e MDA, a atividade da CAT, SOD e GST e a viabilidade celular. Em conclusão, nossos resultados demonstram o seu efeito neuroprotetor do 4-PSQ contra a toxicidade induzida pela ACR.

Palavras-chave: Antioxidante; Anticolinesterásico; Compostos de selênio.

ABSTRACT

Acrylamide (ACR) is a food contaminant formed when carbohydrate-rich foods are heated to high temperatures (above 120°C) and its formation occurs through the Maillard reaction. Several studies have reported that ACR can induce genotoxicity, carcinogenicity, and neurotoxicity. It is suggested that oxidative stress and alterations in the activity of the enzyme acetylcholinesterase (AChE) may be involved in the neurotoxicity caused by ACR, thus compounds with antioxidant and anticholinesterase properties such as 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) can prevent or delay neurotoxicity caused by ACR. Therefore, the aim of this study was to evaluate whether 4-PSQ, an organic selenium compound, has a protective effect against ACR damage induction in *Drosophila melanogaster*. Adult flies (both sexes), between 1 and 3 days old, were divided into 4 groups of 50 flies each: Control; 4-PSQ (25 µM); ACR (5 mM) and ACR (5 mM). + 4-PSQ (25 µM). Flies were exposed to the diet for 4 days according to their respective groups. Flies exposed to ACR had reduced activity rate, locomotor activity, exploratory behavior, and an increase in acetylcholinesterase (AChE) activity. Neurotoxicity was also associated with a marked increase in the reactive species (RS), increased malondialdehyde (MDA) levels, decreased superoxide dismutase (SOD) activity, increased catalase (CAT) activity, increased glutathione S-transferase (GST) activity, and decreased cell viability in the head of flies compared to the control group. This study reveals, for the first time that co-treatment with 4-PSQ in *Drosophila melanogaster* decreased mortality and protected against locomotor deficits, AChE activity, reactive oxygen species (RS) and MDA levels, CAT, SOD and GST activity, and the cell viability. In conclusion, our results show the neuroprotective effect of 4-PSQ against ACR-induced toxicity.

Keywords: Antioxidant; Anticholinesterase; Selenium compounds.

SUMÁRIO

APRESENTAÇÃO

A presente dissertação está organizada em três partes principais. Na primeira parte, encontram-se a INTRODUÇÃO e REVISÃO BIBLIOGRÁFICA, que descrevem a fundamentação teórica desta pesquisa seguidas pelo item OBJETIVOS.

A segunda parte, intitulada MANUSCRITO CIENTÍFICO é composta pelos resultados desta dissertação na forma de manuscrito científico. O manuscrito está estruturado conforme as normas da revista "*Journal of Toxicology and Environmental Health*".

A terceira parte é composta pelas CONSIDERAÇÕES FINAIS que apresenta a discussão sobre os resultados apresentados e o item PERSPECTIVAS, que possui potenciais aplicações dos resultados obtidos. Ao final, encontram-se as REFERÊNCIAS BIBLIOGRÁFICAS citadas na primeira e terceira parte da dissertação. As REFERÊNCIAS BIBLIOGRÁFICAS da segunda parte estão inclusas no manuscrito científico.

1. INTRODUÇÃO

A acrilamida (ACR) é um contaminante alimentar formado durante o processamento térmico dos alimentos como um produto intermediário das reações de Maillard, principalmente através da reação entre o aminoácido asparagina e um açúcar redutor (LOPACHIN; GAVIN, 2008; SANSANO et al., 2017; ZHAO et al., 2017). A formação da ACR ocorre, frequentemente, em alimentos ricos em carboidratos quando expostos a temperaturas acima de 120°C (SANSANO et al., 2017).

É sabido que a ACR possui vários efeitos tóxicos incluindo genotoxicidade, carcinogenicidade e neurotoxicidade (BERTUZZI et al.; 2020; HUANG et al., 2018). Os efeitos tóxicos da ACR afetam o tecido nervoso do sistema nervoso central (SNC) e periférico (SNP), e, dentre as consequências da neurotoxicidade da ACR, incluem-se dano de axônios no SNC e SNP, inibição da liberação de neurotransmissores e alterações na transmissão do impulso nervoso (PALUS; CAŁKA, 2020; WANG et al., 2018). É sugerido que o estresse oxidativo e alterações na atividade da enzima acetilcolinesterase (AChE) possam ser os principais mecanismos envolvidos na toxicidade da ACR (MURRAY, WADDELL, WU, 2020; PRASAD, MURALIDHARA, 2012; ZAMANI et al., 2018). Neste contexto, substâncias com propriedades antioxidantes como os compostos de selênio seriam potenciais agentes para atenuar, prevenir e/ou retardar a toxicidade causada pela ACR (SAVEGNAGO et al., 2013; VOGT et al., 2018).

Diversos estudos demonstraram a capacidade dos compostos de selênio na prevenção de danos oxidativos e proteção contra o desenvolvimento de patologias relacionadas ao estresse oxidativo, possuindo ainda, efeitos neuroprotetores (GHORBEL et al., 2017; DOMINIAK et al., 2017; PATRA et al., 2018; STEFANELLO, et al., 2015). Desta forma, o 7-cloro-4-(fenilselanil) quinolina (4-PSQ), um composto derivado de quinolina contendo selênio em sua molécula, com atividade antioxidante e anticolinesterásica destaca-se como potencial tratamento contra a neurotoxicidade causada pela ACR (COUTO et al., 2019; PINZ et al., 2018; SAVEGNAGO et al., 2013, VOGT et al., 2018).

A mosca da fruta, *Drosophila melanogaster* (*D. melanogaster*) representa uma alternativa no processo de descoberta de novas drogas, tendo em vista que, a maioria dos mecanismos e vias biológicas fundamentais que controlam o desenvolvimento e a sobrevivência foram conservados ao longo da evolução entre humanos e *drosophilas*

(CHENG et al., 2019; JENNINGS, 2011). Nos últimos anos, a *D. melanogaster* tem emergido como um modelo para o estudo de diversas patologias incluindo aquelas que afetam o sistema nervoso, somada a isso, possui múltiplas vantagens como baixo custo de manutenção, tamanho reduzido e curto ciclo de vida quando comparada a outros modelos experimentais (BOLUS et al., 2020; CHENG et al., 2019; NISHIHARA, 2020).

Desta forma, considerando que o estresse oxidativo possa estar relacionado aos principais distúrbios e patologias ligados à exposição a ACR, e, considerando as propriedades antioxidantes e anticolinesterásicas do 4-PSQ, o objetivo do presente estudo foi avaliar o efeito do 4-PSQ frente as alterações comportamentais, oxidativas e colinérgicas induzidas por ACR utilizando como modelo experimental a *D. melanogaster*.

2. REVISÃO BIBLIOGRÁFICA

2.1 Acrilamida

A ACR (prop-2-enamida), é uma molécula orgânica nitrogenada que possui fórmula química C_3H_5NO e cuja estrutura molecular é apresentada nas figuras 1A e 1B. (BERTUZZI et al., 2020; HAMZAHOĞLU; GÖKMEN, 2019). Apresenta-se como um monômero sólido, inodoro, com coloração branca, características cristalinas e possui solubilidade em água, clorofórmio, acetona, dietil éter, etanol, etil acetato e metanol sendo pouco solúvel em heptano (HALFORD; RAFFAN, 2019; IARC, 1994). A presença de um grupo amida polar e uma função vinil em sua estrutura química permitem a sua polimerização (MATOSO et al., 2019).

Figura 1A - Estrutura molecular da acrilamida.

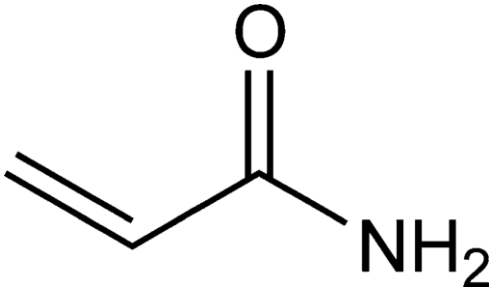
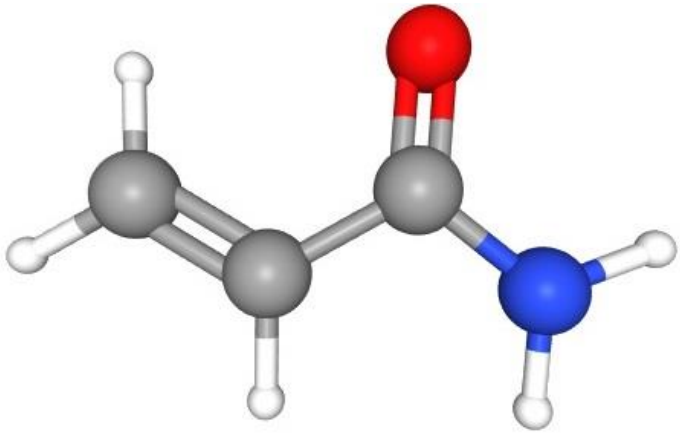


Figura 1B - Estrutura molecular em 3D da acrilamida.



Fonte: Adaptado de National Center for Biotechnology Information. PubChem Database. Acrylamide, CID=6579.

A ACR tem sido utilizada desde 1950 como um intermediário de inúmeras aplicações industriais (DE MEULENAER; MEDEIROS; MESTDAGH, 2016). A sua forma polimerizada é extensamente aplicada como floculante no tratamento de águas industriais, na indústria de papel e celulose, na indústria têxtil, no tratamento de águas residuais e água para consumo, na agricultura como condicionador de solo, como rejunte químico em túneis, poços e esgotos, no processamento de minérios, no refinamento do açúcar, em cosméticos como formador de filme, produtor de espuma e estabilizante e em géis de eletroforese nos laboratórios de pesquisa (ANDERSEN, 2005; COSTA, 2018; DE MEULENAER; MEDEIROS; MESTDAGH, 2016; RALDÚA, 2020).

A polimerização incompleta da ACR pode resultar em quantidades residuais de seu monômero nos produtos finais onde é aplicada e, sabendo que a mesma é utilizada no tratamento da água, a ACR monomérica é reconhecida como um potencial poluente dos recursos hídricos (DE MEULENAER; MEDEIROS; MESTDAGH, 2016; HALFORD; RAFFAN, 2019). Além de suas aplicações industriais e laboratoriais, altos níveis de ACR foram detectados na fumaça do tabaco (MOJSKA; GLELECLŃSKA; CENDROWSKL, 2016).

Em 1997, durante a construção do túnel de Hallandsås, na Suécia, um acidente causou a exposição de trabalhadores e animais a um agente selante contendo monômeros de ACR derivados de sua polimerização incompleta (MATOSO, 2019; TÖRNQVIST; EHRENBERG; HAGMAR, 2000). A aplicação do material selante foi decidida após problemas de vazamento de água enfrentados devido ao caráter poroso do terreno

(TÖRNQVIST; EHRENBERG; HAGMAR, 2000). Entre 5 de agosto a 30 de setembro de 1997, foram aplicadas aproximadamente 1.400 toneladas do selante comercialmente conhecido como Rhoca Gil® (Rhone-Poulec, França) (TÖRNQVIST; EHRENBERG; HAGMAR, 2000).

A água de drenagem foi bombeada para o riacho Vadbäcken, localizado próximo ao túnel (TÖRNQVIST; EHRENBERG; HAGMAR, 2000). Ao final de setembro de 1997, vacas começaram a apresentar paralisia e peixes foram encontrados flutuando mortos (REYNOLDS, 2002; TÖRNQVIST; EHRENBERG; HAGMAR, 2000). Uma análise da água do riacho mostrou altas concentrações de ACR e N-metiloacrilamida, o que demonstrava ser evidente que os monômeros reativos no agente de rejunte vazaram e foram espalhados no meio ambiente (TÖRNQVIST; EHRENBERG; HAGMAR, 2000). Os trabalhadores do túnel que foram expostos à ACR mostraram sinais e sintomas de disfunção do SNP (KOMOIKE, MATSUOKA, 2019).

O acidente ocorrido na Suécia deu início a investigações que demonstraram a presença da ACR em alimentos (TAREKE et al., 2000). Neste estudo, composto por residentes e trabalhadores da área expostos à ACR e grupo controle, foi observado que, inesperadamente, o grupo controle (não fumantes e sem exposição conhecida) possuíam altos níveis de ACR no sangue (TAREKE et al., 2000). Esses achados levaram à hipótese que a ACR poderia estar presente pela exposição dietética (TAREKE et al., 2000)

Neste mesmo estudo, publicado em 2000, ratos alimentados com dieta frita tiveram os níveis de adutos de ACR-hemoglobina mais elevados do que os ratos alimentados com dieta padrão (TAREKE et al., 2000). Em 2002, pesquisadores da Administração Nacional de Alimentos da Suécia e da Universidade de Estocolmo anunciaram que a ACR poderia ser formada em uma ampla variedade de alimentos que foram processados em altas temperaturas (TAREKE et al., 2002).

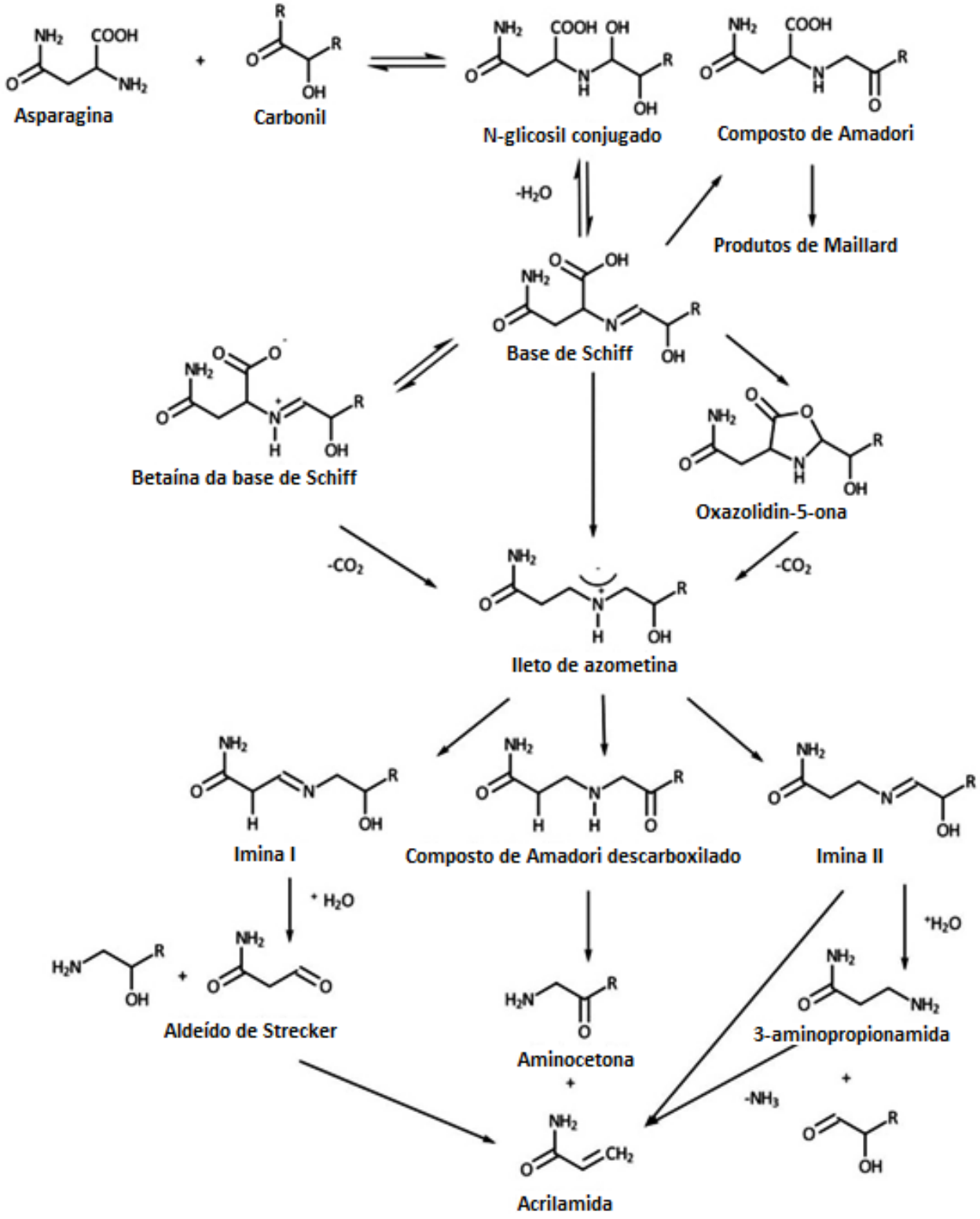
2.2 Formação da Acrilamida em alimentos

Após a descoberta da presença da ACR em alimentos, pesquisas independentes relataram que a reação de Maillard, envolvendo carboidratos com açúcar redutor (principalmente frutose ou glicose) e o aminoácido asparagina, era o principal mecanismo responsável pela formação da mesma (MOTTRAM; WEDZICHA; DODSON, 2002; STADLER et al., 2002). A reação tem início quando os alimentos, com as características

descritas, são expostos a temperaturas acima de 120°C, tendo como exemplo os processos de fritura, torração ou assamento (SANSANO et al., 2017).

O principal mecanismo da formação da ACR (Figura 2) envolve a reação entre um grupo carbonil (preferencialmente um α-hidroxicarbonil) com o aminoácido asparagina, onde, sob condições secas, uma molécula de água é clivada da N-glicosil-asparagina, formando uma base de Schiff após o aquecimento em temperaturas elevadas (DE MEULENAER; MEDEIROS; MESTDAG, 2016; HAMZAHOĞLU; GÖKMEN, 2019). A base de Schiff também pode ser rearranjada para formar compostos de Amadori sob condições aquosas, porém, esses rearranjos são altamente estáveis e não levam a formação de ACR (HAMZAHOĞLU; GÖKMEN, 2019). Os compostos de Amadori são os principais responsáveis pelo desenvolvimento de cores e sabores nos alimentos através da reação de Maillard (HAMZAHOĞLU; GÖKMEN, 2019).

Figura 2 - Mecanismo proposto de formação da acrilamida em alimentos.



Fonte: Adaptado de Hamzahoğlu; Gökmen, 2019.

O segundo passo na formação da ACR é a descarboxilação, que produz um ileto de azometina a partir de uma base de Schiff. O ileto de azometina pode formar imina I através da betaína da base de Schiff, também pode proceder à formação de imina II através da oxazolidin-5-ona. A formação de ACR a partir da imina II pode prosseguir em dois caminhos: (1) a formação de ACR diretamente através da β-eliminação ou (2) a hidrólise de imina II a 3-aminopropionamida seguido pela desaminação para formar ACR. Em outra via, a imina I é hidrolisada a aldeído de Strecker, o qual é também conhecido como um dos precursores diretos da ACR (HAMZAHOĞLU; GÖKMEN, 2019).

Outras rotas foram propostas para a formação da ACR, todavia, a reação de Maillard é tida como a via predominante (HALFORD; RAFFAN, 2019). Além disso, vários fatores afetam a formação da ACR, como temperatura, tempo de aquecimento, umidade, concentração de aminoácidos (asparagina) e presença de açúcares redutores nos alimentos (MATOSO et al., 2019).

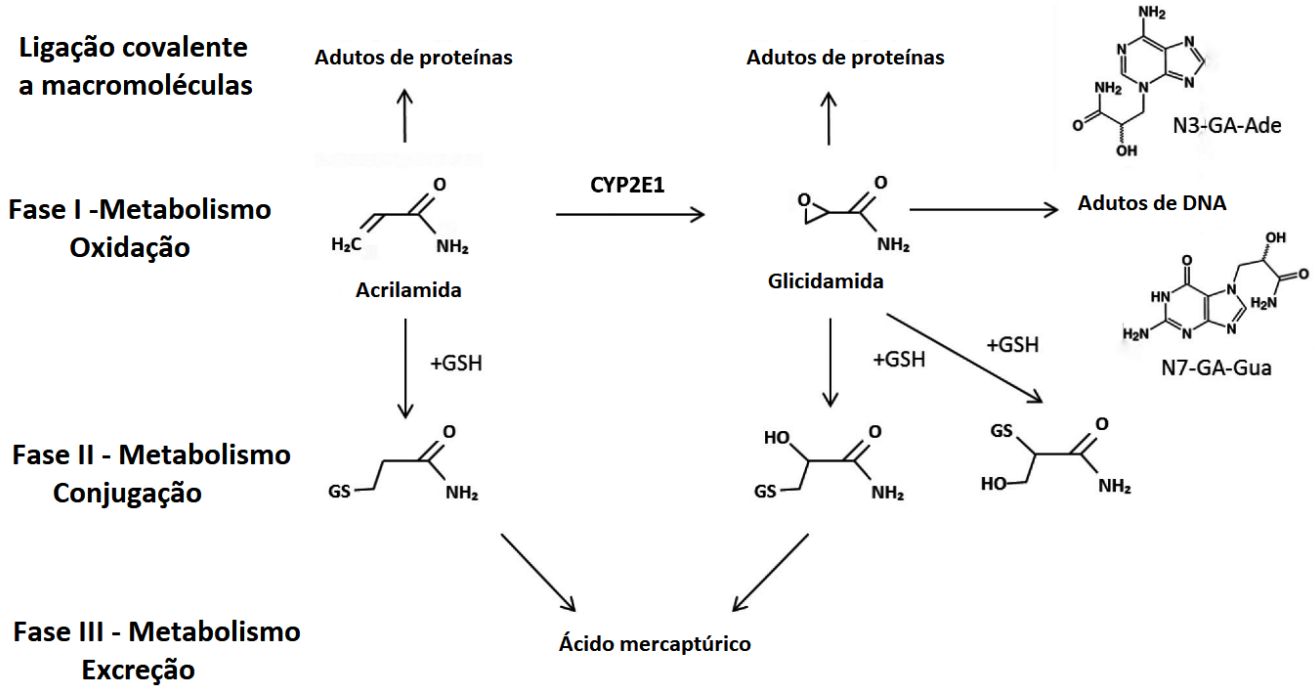
2.2 Toxicologia da Acrilamida, Neurotoxicidade e Estresse Oxidativo

A ACR é uma substância neurotóxica que pode ser absorvida por via oral, dermatológica ou inalatória (SHARMA; KANG, 2020; YU, 2019). Em humanos, após a absorção oral, a ACR é rapidamente distribuída para diferentes tecidos, sendo capaz de atravessar a barreira hematoencefálica, placentária e também pode ser ainda, encontrada no leite materno (ELBLEHI; EYONY; EL-SAYED, 2020; MATOSO et al., 2019).

Após a ingestão, a ACR é metabolizada por duas principais vias, a primeira, é a conjugação da glutationa pela enzima glutationa-S-transferase com o N-acetil-S-(3-amino-3-oxopropil) cisteína e S-(3-amino-3-oxopropil) cisteína como os principais metabólitos, e a segunda via é a oxidação da ACR à glicidamida (GA) pelo citocromo CYP2E1 (FENNEL et al., 2006; OU, et al., 2020). Este metabólito pode ser metabolizado via hidrólise ou conjugação com GSH, pode ainda, reagir com proteínas, hemoglobina ou com moléculas de DNA formando adutos de base de purina (FENNEL et al., 2006; MATOSO et al., 2019). Glicidamida e ACR se ligam covalentemente à valina N-terminal da hemoglobina e formam adutos que servem como biomarcadores da exposição à ACR (OU, et al., 2020). A figura 3 mostra o metabolismo descrito de forma simplificada.

Os metabólitos da ACR são excretados como ácido mercaptúrico pelas vias urinárias e pequenas quantidades também podem ser eliminadas pelas fezes ou ar (MATOSO et al., 2019).

Figura 3 – Via metabólica da acrilamida.



Fonte: Adaptado de Katen; Roman, 2015.

A intoxicação por ACR em humanos por via digestiva é caracterizada por anormalidades na marcha, fraqueza dos músculos esqueléticos, parestesia, transpiração anormal, ataxia, disfunção cognitiva e outros sintomas relacionados à polineuropatia (FARIA et al., 2019, ZHANG et al., 2020). Estudos com animais revelaram que a exposição à ACR induz degeneração dos nervos periféricos e dos axônios da medula oblonga, na coluna anterior e lateral da medula espinhal e no terminal nervoso do cerebelo (ZHANG et al., 2020).

Estes sintomas podem ser explicados pela axonopatia causada pela ACR que é caracterizada por edema axonal próximo aos nódulos de Ranvier resultante da presença de inclusões em neurofilamentos, mitocôndrias e corpos densos (HASCHEK; ROUSSEAUX; WALLIG, 2010). A ACR afeta as fibras sensoriais, particularmente as fibras nervosas mielinizadas de médio e grande diâmetro que inervam os corpúsculos de Pacini e os músculos do fuso muscular aferente primário (HASCHEK; ROUSSEAUX;

WALLIG, 2010). A ACR afeta ainda as membranas e proteínas do citoesqueleto via estresse oxidativo, interrompendo a neurotransmissão em roedores (PRASAD; MURALIDHARA, 2012). Sabe-se ainda que disfunções no sistema colinérgico são características patológicas significativas de neuropatia e a ACR causa pode levar a alterações significativas em marcadores de neurotransmissão, como na atividade da AChE (PRASAD; MUDALIDHARA; 2014). O aumento na atividade da AChE causa uma redução na neurotransmissão colinérgica e, além das funções motoras, afeta outras funções como proliferação celular e promoção de apoptose (PRASAD; MUDALIDHARA; 2014).

Os efeitos tóxicos são observados após a ingestão de doses maiores que 100 mg/kg de peso corporal e doses letais são induzidas após ingestão maior que 150 mg/kg de peso corporal (MATOSO et al., 2019). Após a exposição acima dos níveis seguros, a ACR começa a formar adutos irreversíveis com os aceptores de óxido nítrico (NO) e outros neuromoduladores através da ligação com grupos sulfidril tiolato, reduzindo, transitoriamente a sinalização de moléculas de NO (LOPACHIN; GAVIN, 2014; SENTHILKUMAR et al., 2020). Os compostos α,β-carbonil insaturados de ACR são eletrófilos fracos, o que significa que essas estruturas elétron-deficientes formam preferencialmente adutos (adição de Michael do tipo 1,4) de ligação covalente com nucleófilos fracos, os quais em sistemas biológicos são os grupos sulfidrila que existem no estado nucleofílico aniônico do tiolato (LOPACHIN; GAVIN, 2014).

Isso causa uma diminuição na força sináptica levando a uma interrupção do ciclo da vesícula sináptica, inibindo reversivelmente a função de várias proteínas, como o transportador de membrana da dopamina (recaptação), o fator sensível ao N-etilmaleimida (NEM) (liberação), e o transportador de monoamina vesicular (armazenamento vesicular) (SENTHILKUMAR et al., 2020). Qualquer interrupção em qualquer uma destas propriedades neuronais pode levar à uma toxicidade sináptica, provocando a morte neuronal (SENTHILKUMAR et al., 2020).

Evidências sugerem que os déficits neuronais associados com a intoxicação por ACR resultam de uma progressiva inibição da atividade pré-sináptica no SNC e no SNP onde a ACR, um nucleófilo fraco, forma adutos irreversíveis com grupos nucleofílicos cisteína tiolato que agem como aceptores para NO e outros moduladores redox de atividades de proteínas (LOPACHIN; GAVIN, 2014). A inibição resultante da função da proteína, acoplado a várias funções características anatômicas e funcionais

predisponentes, leva a uma perda acumulativa da atividade terminal nervosa (LOPACHIN; GAVIN, 2014).

Estudos sugerem que a neurotoxicidade induzida pela ACR está possivelmente relacionada com um desequilíbrio entre a função oxidativa e antioxidante, resultando em elevados níveis de espécies reativas de oxigênio intracelular e peroxidação lipídica (PAN et al., 2018). O mecanismo da neurotoxicidade da ACR tem possíveis implicações neuropatogênicas na doença de Alzheimer e outras condições neurodegenerativas que presumivelmente envolvem danos crônicos nos terminais nervosos e a interrupção das vias de sinalização redox, porém, as vias de sinalização de estresse oxidativo envolvidas na neurotoxicidade induzida pela ACR ainda são parcialmente elucidadas (KOMOIKE; MATSUOKA, 2019; LOPACHIN; GAVIN, 2014).

2.4 *Drosophila melanogaster* **como modelo de estudo**

A *Drosophila melanogaster*, pertencente à família Drosophilidae (ordem Diptera) e conhecida popularmente como mosca da fruta, é um importante modelo alternativo e versátil de estudo de diversas patologias humanas (CHENG et al., 2019; DEMIR, 2020; MIRZOYAN et al., 2019). Esta espécie, permitiu que cientistas de diversas áreas ganhassem uma visão profunda da fisiologia de vários organismos, incluindo humanos (DEMIR, 2020). Aproximadamente 13.600 genes na mosca da fruta já foram identificados e, é estimado que aproximadamente 75% dos genes relacionados a doenças em humanos possuem homólogos na *D. melanogaster* e compartilham similaridades em suas funções (ONG et al., 2015; UGUR; CHEN; BELLEN, 2016).

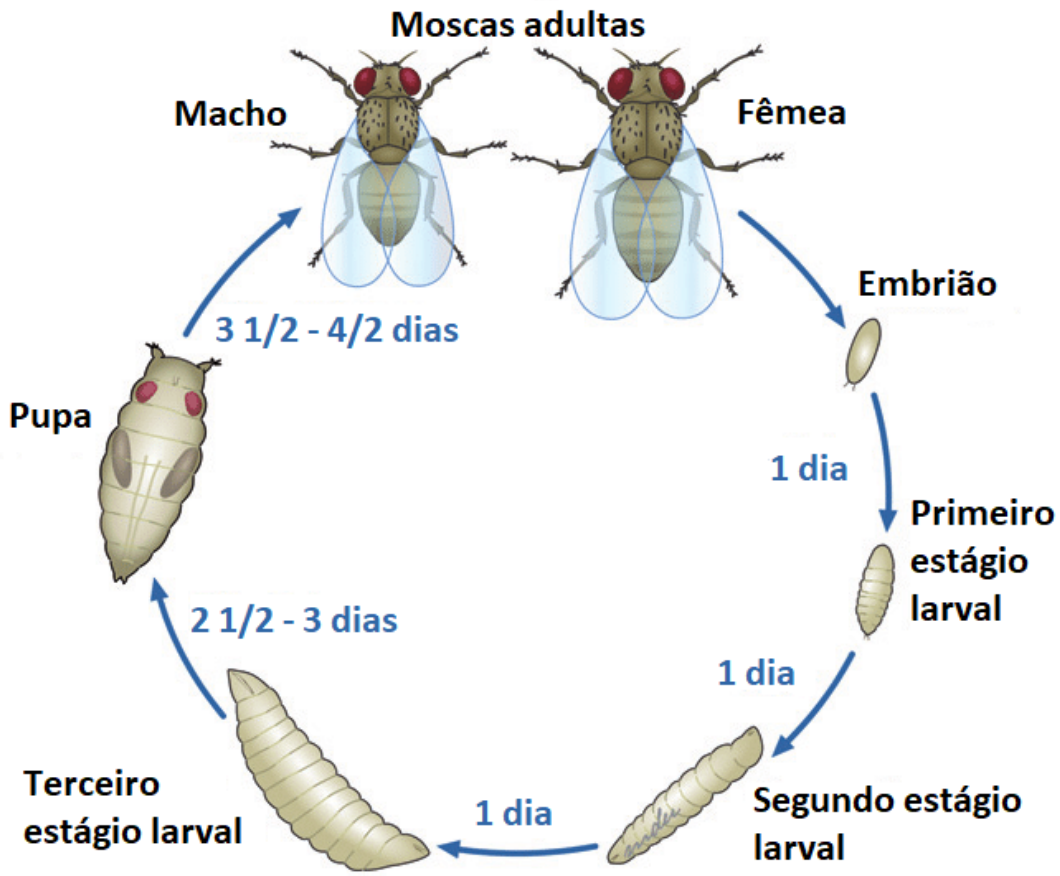
A *D. melanogaster* tem sido utilizada em trabalhos nos diversos campos de pesquisa biológica e médica, incluindo genética, biologia evolutiva, ecologia, fisiologia e patogênese microbiana (DEMIR, 2020). As pesquisas com a mosca da fruta têm avançado ainda na área de patologias incluindo que afetam o sistema nervoso (BOLUS et al., 2020; CHENG et al., 2019; NISHIHARA, 2020).

A mosca da fruta adulta possui estruturas anatômicas fisiologicamente similares ao coração, pulmões, rins, fígado, intestino, trato reprodutivo e cérebro dos humanos (ONG et al., 2015). O sistema nervoso da *Drosophila* é complexo e possui muitos aspectos relacionados ao sistema nervoso humano, incluindo um cérebro, neurônios sensoriais periféricos para propicepção e dor e um cordão nervoso ventral (BOLUS et al.,

2020). O cérebro da mosca adulta possui mais de 100.000 neurônios que formam circuitos discretos e neurópilos que medeiam comportamentos complexos, incluindo ritmos circadianos, sono, aprendizagem e memória, acasalamento, alimentação, agressão, limpeza e vôo (PANDEY; NICHOLS, 2011). Desta forma, a resposta das moscas a muitas drogas que agem no SNC é semelhante aos efeitos observados em mamíferos (PANDEY; NICHOLS, 2011).

Vários métodos podem ser utilizados na mosca da fruta para analisar a neurotoxicidade, como análise de sobrevivência, capacidade locomotora e ensaios bioquímicos (BOLUS et al., 2020; SHAKYA; SIDDIQUE, 2018). Sendo assim, a *D. melanogaster* é um excelente modelo alternativo para estudos neurotoxicológicos (BOLUS et al., 2020).

Figura 4 – Ciclo d vida de *D. melanogaster*



Fonte: Adaptado de ONG et al., 2015.

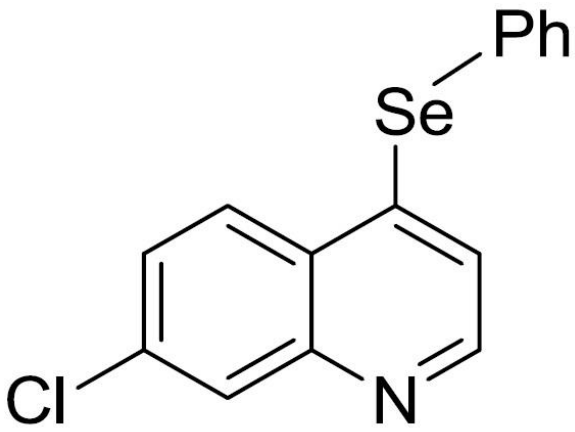
Juntamente com estas vantagens, o baixo custo e facilidade de manutenção, tamanho reduzido e curto ciclo de vida permitem que a mosca da fruta seja um ótimo modelo para estudar mecanismos complexos na pesquisa biomédica (MIRZOYAN et al., 2019, ONG et al., 2015).

2.5 7-cloro-4-(fenil selanil) quinolina (4-PSQ)

É sabido que os compostos de selênio são conhecidos por possuírem importante papel nos sistemas de defesa antioxidante do organismo e, consequentemente, na prevenção/tratamento de patologias relacionadas ao estresse oxidativo (GHORBEL et al., 2017; DOMINIAK et al., 2017; PATRA et al., 2018; SENTKOWSKA; PYRZYŃSKA, 2019). Da mesma forma, as quinolinas, compostos nitrogenados heterocíclicos constituídos por um anel benzeno ligado à pirimidina são importantes drogas usadas no tratamento de várias doenças/desordens (HUSSAINI, 2016). Diversos estudos comprovaram a ação antimicrobiana, antimalárica, anti-inflamatória, neuroprotetora e antiviral destes compostos (HUSSAINI, 2016).

Neste contexto, destaca-se o 4-PSQ, um composto derivado das quinolinas com selênio em sua molécula, que apresenta diversos estudos comprovando variadas propriedades farmacológicas, tais como: atividade antioxidante (LUCHESE et al., 2020; PINZ et al., 2018; VOGT et al., 2018, SILVA et al. 2021), anticolinesterásica (COUTO et al., 2019; PINZ et al., 2018), ação antinoceptiva e anti-inflamatória (PINZ et al., 2016). A síntese do 4-PSQ foi descrita pela primeira vez em 2013 (Savegnago et al., 2013) onde, neste mesmo trabalho, foi evidenciada sua capacidade antioxidante *in vitro*.

Figura 5 – Estrutura molecular do 4-PSQ.



Outros autores relataram que o 4-PSQ foi capaz de reduzir os níveis de espécies reativas e peroxidação lipídica, restaurar a atividade das enzimas SOD e CAT em *D. melanogaster* expostas à rotenona durante 7 dias (COUTO et al., 2019), restaurar a homeostase redox e os marcadores bioquímicos de danos hepáticos e renais em tecidos de ratos idosos expostos ao 4-PSQ (7 dias) (LUCHESE et al., 2020) e exercer efeito ansiolítico (dose aguda) em camundongos machos adultos (REIS et al., 2017).

O 4-PSQ demonstrou-se capaz de aumentar a sobrevivência, reduzir os níveis de glicose, triglicerídeos e de TBARS e normalizar as atividades da SOD e CAT em um modelo crônico de exposição (10 dias) à dieta hiperglicídica em moscas *D. melanogaster* (SILVA et al. 2021), proteger contra os danos em aprendizado, memória e ansiedade em um modelo de doença de Alzheimer em camundongos que foram tratados com 4-PSQ por 14 dias (PINZ et al., 2018), restaurar a capacidade cognitiva causada pelo envelhecimento, modulando a plasticidade sináptica o sistema colinérgico e os níveis de colesterol em ratos machos expostos ao 4-PSQ (7 dias) (BARTH et al. 2019).

O composto foi capaz de reduzir o estresse oxidativo renal induzido por oxaliplatina em camundongos (MOTTA et al., 2021), apresentou efeito ansiolítico sem causar danos locomotores em camundongos machos (PALTIAN et al., 2020) e reduziu o comportamento ansioso e danos cognitivos causados por oxaliplatina em camundongos (REIS et al., 2020).

3. JUSTIFICATIVA

Nas últimas décadas, diversos estudos têm demonstrado os efeitos tóxicos que podem ser causados pela exposição à ACR assim como as patologias que por ela podem ser desencadeadas, sendo que, parte destes danos, estão relacionados ao estresse oxidativo e alterações na atividade da enzima acetilcolinesterase. Dentro deste aspecto, é necessário investigar substâncias com propriedades antioxidantes e anticolinesterásicas na tentativa de proteger ou amenizar os danos relatados.

Portanto, sabendo-se dos efeitos antioxidantes e anticolinesterásicos do 4-PSQ, um composto quinolínico de selênio com comprovada eficácia na melhora de déficits comportamentais, melhora nas defesas antioxidantes, e efeito anticolinesterásico, esse trabalho visa aumentar a compreensão sobre o 4-PSQ através do estudo sobre seu efeito frente as alterações motoras e bioquímicas associadas à exposição a ACR em *D. melanogaster*.

4. OBJETIVOS

4.1 Objetivo geral

- Avaliar o efeito do 7-cloro-4-(fenilselanil) quinolina frente as alterações comportamentais, oxidativas e colinérgicas induzidas por ACR utilizando como modelo experimental a *D. melanogaster*.

4.2 Objetivos específicos

- Observar a taxa de sobrevivência das moscas após a exposição aguda à ACR e ao tratamento com 4-PSQ;

- Verificar as alterações motoras em *D. melanogaster* após a exposição à ACR e ao tratamento com 4-PSQ;

- Avaliar se a ACR induz alterações na atividade da AChE e se o 4-PSQ exerce efeito anticolinesterásico;

- Verificar o comportamento dos indicadores de dano oxidativo na cabeça das moscas expostas à ACR e comparar se o 4-PSQ exerce efeito protetor contra esses danos;

- Avaliar a viabilidade celular na cabeça de *D. melanogaster* expostas à ACR e 4-PSQ.

5. MANUSCRITO CIENTÍFICO

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de manuscrito científico. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas, encontram-se no próprio manuscrito. O manuscrito está disposto na forma que será submetido à revista "*Journal of Toxicology and Environmental Health*".

Manuscrito:

"7-chloro-4-(phenylselanyl) quinoline protects against behavioral, oxidative and cholinergic changes induced by acrylamide exposure in *Drosophila melanogaster"*

Francielli Polet Almeida, Márcia Rósula Poetini, Elize Musachio, Dieniffer Espinosa Janner, Vandreza Cardoso Bortolotto, Nathalie Savedra Gomes, Diego Alves, Gustavo Guerra, Marina Prigol

7-chloro-4-(phenylselanyl) quinoline protects against behavioral, oxidative and cholinergic changes induced by acrylamide exposure in *Drosophila melanogaster*

Francielli Polet Almeida[a], Márcia Rósula Poetini[a], Elize Musachio[a], Dieniffer Espinosa Janner[a], Vandreza Cardoso Bortolotto[a], Nathalie Savedra Gomes[b], Diego Alves[c], Gustavo Guerra[a], Marina Prigol[a*]

[a]Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas- LaftamBio Pampa- Universidade Federal do Pampa (UNIPAMPA), Campus Itaqui – Rua Joaquim de Sá Britto, s/n – Bairro: Promorar, Itaqui, Rio Grande do Sul, CEP 97650-000, Brazil.
[b]Laboratório de Análises Químicas Ambientais e Toxicológicas (LAQAT), Universidade Federal Do Pampa, Uruguaiana, RS, Brazil
[c]Laboratório de Síntese Orgânica Limpa, LASOL, Centro de Ciências Químicas, Farmacêuticas e de Alimentos (CCQFA), Universidade Federal de Pelotas, Campus Universitário, S/N - Prédio/Bloco: 30 e 32, Capão do Leão, Rio Grande do Sul, CEP 96160-000, Brazil.

Declarations of interest: none.

[*]Corresponding author:
Marina Prigol
Universidade Federal do Pampa, Campus Itaqui
Rua Luiz Joaquim de Sá Britto, s/n - Bairro: Promorar, Itaqui, Rio Grande do Sul, Brasil, CEP 97650-000
Orcid ID: https://orcid.org/0000-0002-9724-7469
E-mail addresses: marinaprigol@unipampa.edu.br (M. Prigol)

Abstract

Acrylamide (ACR), a neurotoxic substance, is a food contaminant generated during high-temperature cooking of carbohydrate-rich foods. The present study aimed to evaluate the protective effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) on oxidative, enzymatic, and behavioural parameters against ACR-induced neurotoxicity in *Drosophila melanogaster* (*D. melanogaster*). Adult flies, 1-3 days old were divided into 4 groups of 50 flies each: Control; 4-PSQ (25 μM); ACR (5 Mm) and ACR (5 Mm) +4-PSQ (25 μM). The flies were exposed to the diet for 4 days according to their respective groups. Flies fed with ACR presented a reduced survival rate, locomotor activity, and an increase in AChE activity. ACR-induced neurotoxicity has also been associated with an increase in reactive species (RS) production, an increase in malondialdehyde (MDA) levels, a decrease of superoxide dismutase (SOD) activity, increase of catalase (CAT) activity, increase in glutathione S-transferase (GST) activity, and decrease in cell viability of flies' heads. Co-exposure to 4-PSQ increased survival rate, improved locomotor activity, and maintained AChE activity close to the control group. The co-exposure also normalized ROS and MDA levels, SOD activity, CAT activity, GST activity, and the cell viability in the flies' heads. Based on our results, 4-PSQ exhibited neuroprotective effects, probably due to its antioxidant and anticholinesterasic properties, suggesting the association of both effects with the protection against locomotor impairments and oxidative damage. Therefore, the 4-PSQ is a therapeutic potential for the prevention against ACR-induced neurotoxicity.

Keywords: Antioxidant; Neuroprotective; Oxidative Stress; Selenium compounds.

1. Introduction

Acrylamide (2-propenamide) (ACR) is a significant food contaminant that has his formation during heat treatment as a result of the Maillard reactions, mostly due to the reactions between the free amino acid asparagine and reducing sugars (mainly glucose and fructose) (Chang et al., 2020, Mottram et al., 2002; Nomi, Otsuka, 2020, Stadler et al., 2002). ACR can be formed when frying, roasting, or baking carbohydrates rich-foods at temperatures above 120°C (Sansano et al., 2017).

Studies *in vitro* and *in vivo* have shown that ACR can induce oxidative stress, and neurotoxicity (Chen et al., 2017, Duan et al., 2015, Huang et al., 2018, Matoso et al., 2019, Zamani et al., 2017). ACR neurotoxicity affects the central and peripheral nervous system, resulting in impairment of sensory and motor functions (Prasad, Muralidhara, 2014). It is suggested that oxidative stress and acetylcholinesterase (AChE) alterations play a crucial role in the toxicity induced by ACR (Prasad, Muralidhara, 2012, Zamani et al., 2017). Thus, substances with antioxidant properties such as selenium compounds would be potential agents to mitigate, prevent and/or delay the toxicity caused by ACR (Savegnago et al., 2013; Vogt et al., 2018).

A wide variety of selenium compounds are studied due to their ability to prevent oxidative damage and protect against the development of pathologies related to oxidative stress (Ghorbel et al., 2017, Dominiak et al., 2017, Patra et al., 2018). Similarly, several molecules containing quinoline, an organic compound characterized by a benzene ring fused to the pyridine ring, are drugs that have clinical significance (Hussaini, 2016).

7-chloro-4- (phenylselanyl) quinoline (4-PSQ), a compound derived from quinoline that has selenium in its molecule, has several pharmacological properties. This compound presented an modulated the oxidative stress in different tissues of aged rats (Luchese et al., 2020), showed ability to restore the cognitive impairment caused by aging in male rats (Barth et al., 2019), presented anxiolytic effect without cause motor impairment in male mice (Paltian et al., 2020), reduced anxious behavior and cognitive impairment caused by oxaliplatin in mice (Reis et al., 2020), protected against learning, memory impairment, and anxiety in an Alzheimer's disease model induced by amyloid β-peptide in mice (Pinz et. al, 2018) and presented neuroprotective, antioxidant and

anticholinesterasic effect in a Parkinson's-like disease model induced by rotenone in *D. melanogaster* (Couto et al., 2019).

Drosophila melanogaster (*D. melanogaster*) is an alternative and versatile model of study, which shows high levels of similarity with human physiology and anatomical structures (Cheng et al., 2019). The research with *D. melanogaster* emerged as a potential model for the study of several human pathologies including those that affect the nervous system (Bolus et al., 2020, Cheng et al., 2019, Nishihara, 2020). The studies using *D. melanogaster* are supported by the fact that the *Drosophila* nervous system is complex and has many aspects of the human nervous system including a brain, peripheral sensory neurons for proprioception and pain, and a ventral nerve cord (Bolus et al., 2020). Multiple methods can be used in *Drosophila* to analyse neurotoxicity, such as lifespan analysis, locomotor performance, and biochemistry assays (Bolus et al., 2020; Shakya, Siddique, 2018). Thus, *D. melanogaster* is an excellent non-mammalian alternative model for neurotoxicological study (Bolus et al., 2020).

Therefore, considering that oxidative stress may be related to the main disorders and pathologies associated with exposure to ACR, and considering the antioxidant and anticholinesterasic properties of 4-PSQ, the objective of the present study was to evaluate the effects of 4-PSQ on behavioural, oxidative and cholinergic changes induced by ACR using *D. melanogaster* as an experimental model.

2. Materials and Methods

2.1 Chemicals

ACR (Figure 1) (> 99% purity; Sigma Aldrich, St. Louis, MO, USA) was initially diluted in distilled water to obtain the required concentrations. The distilled water served as a negative control. 4-PSQ (Figure 2) was prepared and characterized following the method previously described by Savegnago et al. (2013). Analysis of the ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra in full agreement with its assigned structure showed analytical and spectroscopic results. Gas chromatography-mass spectrometry (GC/MS) had determined the chemical purity of 4-PSQ (99.9%). 4-PSQ was dissolved in ethanol. All other reagents used in this study were of analytical grade and obtained from the laboratory of the Federal University of Pampa, Campus Itaqui.

2.2 *Drosophila melanogaster (D. melanogaster)* stock and culture

D. melanogaster (wild-type, Harwich strain) was obtained from the National Species Stock Center (Bowling Green, Ohio, USA). The flies were maintained in a BOD incubator at 25°C (± 2°C), 60% relative humidity in a 12:12 h light-dark cycles and fed a standard diet (76,59% corn flour, 8.51% wheat germ, 7,53% sugar, 7,23% milk powder, 0.43% salt and 0.08% methylparaben as an antifungal agent. All experiments were carried out with the same strain.

2.3 Experimental Protocol

2.3.1. ACR exposure and 4-PSQ treatment

D. melanogaster (both sexes) 1-3 days old were divided into four groups of 50 flies each: (1) Control, (2) 5 mM ACR (3) 25 μM 4-PSQ, and (4) 5 mM ACR + 25 μM 4-PSQ. Flies were exposed to a diet for 4 days according to their respective groups. The control group received distilled water.

To determine the ACR concentration used in this work, *D. melanogaster* were divided into four groups of 50 flies each and exposed to ACR (diluted in distilled water) at varying concentrations (1 mM; 2 mM; 5 mM and 10 mM) in the diet for 4 days (50 per replicate; 3 replicates per group). Distilled water was used as control. The concentration chosen after the curve was 5 mM. The 4-PSQ concentration (25 μM) was based on a previous study of Couto et al. (2019) which showed that 4-PSQ did not cause signals of toxicity in flies.

2.4 *In vivo* assays

2.4.1 Survival

Flies were collected from each group, divided into tubes of 50 flies, and transferred to fresh food containing the respective treatment groups. The survival assays were performed with the number of live flies counted daily until the end of the

experimental period (4 days). The total number of survival flies reflects the sum of three separate experiments (50 flies/group).

2.4.2 Negative geotaxis - Climbing test

The locomotor capacity was calculated using the negative geotaxis assay according to the method described by Araujo et al. (2015). After treatment of 4 days, the flies were individually placed in falcon tubes (15 cm length and 1.5 cm in diameter) and immobilized on ice for 1-2 min. After a brief recovery period (10 minutes), flies were carefully tapped to the bottom. Climbing time was determined by the time spent by each fly to achieve a height of 8 cm. This assay was repeated five times at 1-minute intervals and 6 flies from each group were separated to be evaluated. The data for this analysis was according to the average time of each fly. For this analysis, we performed three independent experiments and a total of 72 flies (6 per group) were used.

2.4.3 Open field

To evaluate the exploratory behavior, 5 flies were used in each group; every fly was assessed individually after submitted into a Petri dish divided by squares (1 cm x 1 cm) as described by Hirth (2010). The flies were anesthetized on ice and individually transferred to the Petri dish. After 10 minutes of recovery, fly activity and movement were evaluated by the resulting trajectory for the time (60 s). The total number of squares was calculated according to the number of crossed squares by the flies. For this analysis, we performed four independent experiments and a total of 80 flies (5 per group) were used.

All behavioural studies were performed at 25°C (± 2°C), under adequate daylight conditions, and between 9 am to 4 pm.

2.5 *Ex vivo* assays

2.5.1 Homogenate preparation

After the treatment protocol, flies were anesthetized by freezing in ice, and immediately *D. melanogaster* heads were separated from bodies with a sharp edge. The heads were homogenized separated in HEPES buffer (20 mM, pH 7.0) according to each protocol analysis. The supernatant was removed after centrifugation, and used for biochemical assays. All experiments were performed in triplicate.

2.5.1.1 Determination of AChE activity

AChE activity was determined according to the method proposed by Ellmann et al. (1961) that is based on the production of thiocholine by the hydrolysis of acetylcholine (ACh). Twenty flies' heads per group were homogenized in 200 µL of 20 mM HEPES buffer (pH 7.0) and centrifuged at 1000 xg for 5 minutes at 4°C. The reaction mixture was prepared with buffer (0.25 M KPI, pH 8.0, and dithiobis-2-nitrobenzoic acid - 5 mM DTNB). An acetylthiocholine solution (7.25 m; 2.1 mg/mL) was added to the supernatant and the reaction was monitored and performed for 2 minutes at 412 nm. The enzymatic activity was expressed as nmol of hydrolyzed substrate/min/mg protein. For this analysis, we performed six independent experiments (n= 20 heads) for each treatment group.

2.5.1.2. Oxidative stress markers

Lipid peroxidation (LPO) was measured as thiobarbituric acid reactive substances (TBARS) and performed with modifications using the method described by Ohkawa et al. (1979). Fifteen flies' heads in each group were homogenized in 150 µL of HEPES buffer and centrifuged at 1000 x g for 10 min at 4°C. The supernatant was removed and it was added the thiobarbituric acid (TBA 0.8%, pH 3.2), acetic acid/HCl (20%, pH 3.5), and sodium dodecyl sulfate (SDS 8.1%). The samples were incubated at 95°C for 2 hours. Absorbance was determined spectrophotometrically at 532 nm. TBARS levels were normalized by protein concentration and were expressed as nmol malondialdehyde

(MDA)/mg tissue. For this analysis, we performed six independent experiments (n= 15 heads) for each treatment group.

RS generation was measured based on the oxidation of the non-fluorescent 2′,7′-dichlorofluorescein diacetate (H_2DCF-diacetate) to the fluorescent 2′,7′-dichlorofluorescein (DCF). To quantify the generation of RS, fifteen heads per group were homogenized in 150 µL of 10 mM Tris buffer (pH 7.4). The homogenate was centrifuged at 1,000 x g at 4°C, and the supernatant was removed for the 2',7'-dichloro fluorescein diacetate (DCF-DA) oxidation assay, as well as a general oxidative stress index according to the protocol of Perez-Severiano et al., (2004). The fluorescence emission resulting from the oxidation of DCF-DA was monitored after one hour, the emission of 530 nm and excitation 488 nm. For this analysis, we performed four independent experiments (15 heads per group). The values of all groups were normalized against the values of the control and the RS generation was *expressed* as a percentage of *the control* DCF formation in arbitrary units (AU).

2.5.1.3 Antioxidant enzymes

SOD activity was measured by monitoring the inhibition of quercetin auto-oxidation (Kostyuk, Potapovich, 1989) with modifications (Franco et al., 2009). Twenty flies' heads from each group were homogenized in 200 µL of HEPES buffer and centrifuged at 1000 x g at 4°C for 10 min. The samples were diluted at 1:10. The solution was prepared containing 0.025 M KPi buffer, 0.1 mM EDTA (pH 10.0) and N,N,N,N-tetramethylethylenediamine (TEMED). The reaction was started by adding 0.15% quercetin dissolved in dimethylformamide. The reaction was monitored for 2 min at 406 nm. The results were expressed as the amount of protein required to inhibit 50% of quercetin auto-oxidation. For this analysis, we performed four independent experiments (20 heads per group). The enzymatic activity was expressed as U/mg protein.

Catalase activity was determined following the method proposed by Aebi (1984), with modifications in which the decomposition of peroxide is estimated spectrophotometrically. Twenty flies' heads from each group were homogenized in 200 µL of HEPES buffer and centrifuged at 2000 x g at 4°C for 30 min. Potassium phosphate buffer solution (0.25 M/EDTA 2.5 mM, pH 7.0), 30% hydrogen peroxide (H_2O_2) and Triton X-100 was prepared. The solution was added to the supernatant and absorbance

was measured for 2 min at 240 nm immediately after adding hydrogen peroxide. For this analysis, we performed four independent experiments (20 heads per group). The catalase activity was expressed as U/mg protein.

The GST activity was determined by the method of Habig et al. (1974) monitoring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with glutathione (GSH). Twenty flies' heads in each group were homogenized in 200 µL of HEPES buffer. The supernatant was removed and the samples (30 µL of head) were prepared containing buffer 0.25M/EDTA (2.5 mM, pH 7.0), distilled water, and 100 mM GSH. CDNB 50 mM was added as a substrate and monitored at 340 nm for 2 min. The enzymatic activity was expressed as nmol CDNB conjugated $min.mg^{-1}$ protein. For this analysis, we performed four independent experiments (n= 20 heads) for each treatment group.

2.5.1.4 Determination of mitochondrial activity by MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and cell viability by Resazurin assay

The MTT assay was performed according to Hosamani et al. (2010) with some modifications. This assay is based on the reduction of MTT by the mitochondrial dehydrogenases to purple-colored formazan crystals. Around 60 heads of flies per group were homogenized in Tris buffer (250 mM, pH 7.4) and, the supernatant was centrifuged at 2500 xg for 5 min at 4°C. Mitochondria were isolated by centrifuging the post-nuclear supernatant at 12000 xg for 10 min at 4°C. The pellet was washed with buffer HEPES-mannitol-sucrose, and then the pellet was re-suspended in 200 µL of the same buffer that was incubated for 30 min at 37°C and centrifuged at 10000 xg for 5 min. DMSO (200 µL) was added and the sample was incubated for 30 min at 37°C. Finally, 200 µL of DMSO was added and the supernatant was centrifuged at 3000 xg for 20 min. Absorbance was measured using a microplate reader at 540 nm. The results were expressed as a percentage of the control. The values of all groups were normalized against the values of the control, and the cell viability values were presented as the percentage of the cells surviving in comparison to the control group. For this analysis, we performed six independent experiments (around 60 heads per group).

The Resazurin assay was determined based on the intracellular reduction of resazurin to resorufin by metabolically active cells (Franco et al., 2009). Ten flies' heads per group were homogenized in 1 mL of 20 mM Tris buffer (pH 7.0) following

centrifugation at 999g for 10 min at 4°C. The supernatant was incubated in 96-well plates with 180 µL of 20 mM Tris buffer (pH 7.0) and 10 µL of resazurin for 1 h. After this period, the fluorescence emission was read at 573 nm. The results are expressed as a percentage of the control group. For this analysis, we performed six independent experiments (10 heads per group).

2.5.1.5 Protein determination

The protein concentration of the head homogenates (10 µL head sample, dilution 1:10) was measured by the method of Bradford (1976) using bovine serum albumin as the standard. The sample was added to 190 µL of Bradford reagent followed by a 5 min. incubation at room temperature. The absorbance was measured at 595 nm using a spectrophotometer.

2.6 Statistical studies

Statistical analysis of the results and the graphical representation was obtained by the software GraphPad Prism 8. Lifespan measurement was determined by comparing the survival curves with a log-rank (Mantel-Cox test). The LC50 was calculated using probit analysis in the software Excel. All data were analyzed for normal distribution by the Shapiro-Wilk normality test, and the homogeneity of the data was verified through Bartlett's test. Statistical analyses were performed by the One-way analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test. Differences between groups were applied when $p < 0.05$.

3. Results

3.1 Acrylamide exposure reduced the survival rate of *D. melanogaster*

Flies exposed to ACR 1mM, 2 mM, 5 mM, and 10 mM had a decrease in survival rate after 4 days of exposure compared to the control group ($p<0.05$), One-way ANOVA,

(Fig. 3A). The survival rate of *D. melanogaster* after 4 days of exposure to control, ACR 1 mM, 2 mM, 5 mM, and 10 mM was 93,95%; 87,93%; 67,12%; 39,33%, and 5,33%, respectively. While ACR at lower concentrations (1 mM and 2 mM) did not cause any statistically significant mortality, the highest concentration (10 mM) caused the death of almost all flies. LC50 value for ACR determined in this study was 3.02 mM. Given this finding, all subsequent experiments utilized ACR concentrations of 5 mM.

3.2 4-PSQ attenuated the decrease in survival of ACR-exposed flies

No significant difference ($p > 0.05$) was observed in the survival rate of the control group (93.92%) compared to the 4-PSQ group (95.24%). The co-exposure of 4-PSQ (25 μM) with ACR significantly increased the survival rate (75.06%) compared to the ACR group (58.40%), despite that, the survival of ACR+4-PSQ group was significantly lower (75.06%) than the control group, demonstrating a partially protective effect of 4-PSQ (Fig. 3B).

3.3 Effects of 4-PSQ in the locomotor performance (negative geotaxis and open field test) of ACR exposed flies

D. melanogaster exposed to ACR presented a decrease in the locomotor performance increasing significantly the time to reach the target distance (884.83%) compared to the control group (Fig. 4A). Besides, ACR+4-PSQ group revealed no significant differences compared to the control group. All the above results suggested that 4-PSQ protected flies from the locomotor impairment induced by ACR ($F = 1.606$; $P \leq .0.0001$; One-way ANOVA). Along with a decreased climbing rate, ACR exposure lowered the locomotor and exploration activity of the flies compared to the control group (Fig. 4B). However, when 4-PSQ was administered concomitantly with ACR, there was a significant increase in the trajectory traveled by the flies, showing that the 4-PSQ partially prevented the damage in the locomotor functions in ACR-induced toxicity flies ($F = 5.044$; $P \leq .0.0001$; One-way ANOVA).

3.3 4-PSQ ameliorated the AChE increase in the ACR-exposed *D. melanogaster*

ACR-treated flies showed a significant increase in the AChE activity as compared to control. ACR+4-PSQ-treated flies significantly attenuated the increase in the AChE activity as compared to the ACR-treated flies (Fig. 5). However, no significant differences were found in the AChE activity after 4-PSQ treatment in *D. melanogaster* as compared to control ($F = 0.2043$; $P \leq .0.0015$; One-way ANOVA).

3.4 Oxidative stress markers

Exposure of flies to ACR caused a significant increase in MDA levels in *D. melanogaster* heads compared to control. No significant change in MDA levels was observed in flies treated with 4-PSQ compared to control. ACR+ 4-PSQ group exhibited a significant decrease in the MDA levels compared to the ACR group suggesting a significant protection (Fig.6B) ($F = 0.2221$; $P \leq .0.0001$; One-way ANOVA).

ACR caused a significant increase in the level of RS in flies' heads compared to the control group. ACR+4-PSQ significantly reduced RS production (Fig. 6A). These results suggest that 4-PSQ modulate the oxidative stress caused by ACR exposure ($F = 1.782$; $P \leq .0.0001$; One-way ANOVA).

3.5 Antioxidant enzymes

The exposure of flies with ACR resulted in a significant inhibition in the activity of SOD in flies' heads. The treatment with 4-PSQ provided significant protection against the inhibition of SOD activity induced by ACR (Fig. 7A) ($F = 0.9343$; $P \leq .0.0067$; One-way ANOVA).

D. melanogaster exposed to ACR demonstrated a significant increase in the activity of CAT, while no change was observed in the 4-PSQ group compared to the control. ACR + 4-PSQ group showed a significant increase in catalase activity compared to the ACR group (Fig. 7B) ($F = 2.016$; $P \leq .0.0017$; One-way ANOVA).

Administration of ACR caused a significant increase in GST activity when compared to the control group. However, the treatment with 4-PSQ significantly attenuated the increase of GST activity caused by ACR when compared with the control group (Fig. 7C) ($F = 1.871$; $P \leq .0.0035$; One-way ANOVA).

3.6 Cell viability assays

MTT assay results showed that exposure to ACR significantly reduced cell viability compared to the control group. ACR+4-PSQ significantly inhibited ACR-induced cell death (Fig. 8A) ($F = 1.281$; $P \leq .0.0001$; One-way ANOVA).

The Resazurin assay results (Fig. 8B) show that ACR exposure caused a significant decrease in cell viability compared to the control group. However, ACR+4-PSQ significantly mitigated the ACR cell toxicity ($F = 1.051$; $P \leq .0.0002$; One-way ANOVA).

4. Discussion

The present study was aimed at analyzing the survival rate, locomotor ability, and biochemical parameters of *D. melanogaster* exposed to 4-PSQ against ACR-induced neurotoxicity. It is known that oxidative stress plays an important role in ACR-induced neurotoxicity (Prasad, Muralidhara, 2012; Zhao et al., 2017), and antioxidant substances, like selenium compounds, demonstrate a protective effect against neurotoxicity induced by RS (Hafiz et al., 2020). Therefore, the 7-chloro-4-(phenylselanyl) quinoline (4-PSQ), a quinoline-derived compound containing selenium in its molecule, with several pharmacological properties, including potential antioxidant activity (Savegnago et al., 2013; Couto et al., 2019; Vogt et al., 2018) becomes an important substance to be tested against ACR neurotoxicity. However, no studies, up until now, have examined 4-PSQ for its neuroprotective role in the ACR-induced neurotoxicity model. This study reveals, for the first time that ACR+4-PSQ in *D. melanogaster* decreased mortality and prevented locomotor impairment, AChE activity, RS and MDA levels, CAT, SOD and GST activity, and the cell viability suggesting its potential neuroprotective role against ACR-induced neurotoxicity.

Based on the mortality of flies exposed to different concentrations of ACR, the concentration of 5 mM was chosen for further studies. The findings of this study confirm certain outcomes observed in the prior survival studies where ACR exposure was associated with increased mortality in organisms, such as *C. elegans* (Murray et al. 2020), rats (Elblehi et al., 2020, Liu et al., 2021); zebrafish (Park et al., 2021) and *D. melanogaster* (Senthilkumar, et al.,2020).

The exposure of adult flies to the dose of ACR (5mM) resulted in significant mortality after 4 days. The survival rate of control (93.92%) was significantly higher than the ACR exposed group (58.40%). However, when 4-PSQ was administered concomitantly with ACR, the flies had shown significant improvement in their survival rate. Our results agree with data obtained by Couto et al. (2019) and Silva et. al (2021), which demonstrated that 4-PSQ increased the survival rate of flies treated with rotenone and a high sucrose diet, respectively.

It has been known that ACR is a neurotoxic substance and ACR-induced neurotoxicity, in addition to increased mortality, is characterized by locomotor function impairments (Park et al., 2021). The results of the present study showed that ACR induced a marked decrease in locomotor activities in negative geotaxis and open field tests. These data are consistent with previous studies that examined locomotor activity after exposure to ACR in *D. melanogaster* (Senthilkumar, et al. 2020) and other organisms such as zebrafish (Faria et al., 2019) and rats (Liu et al., 2021). Similar to previous studies which 4-PSQ prevented the locomotor impairment of flies exposed to rotenone (Couto et al., 2019) and partially prevented the locomotor damage of aging rats (Barth et al., 2019). Our study demonstrated that the ACR+4-PSQ could almost completely protect *D. melanogaster* against ACR-induced behavioural impairment. These findings are strongly associated with a regulation of the AChE activity of the group ACR + 4-PSQ compared to the ACR group.

Locomotor impairments may be associated with AChE activity alterations. In our study, AChE activity was significantly increased in the head of ACR-exposed flies. Previous studies have shown that ACR increases the activity of AChE (Aydın, 2017, Prasad, Muralidhara, 2012). AChE is a key biological component of cholinergic function and membrane, and it is known to help maintain membrane integrity during synaptic transmission and conduction (Prasad, Muralidhara, 2012). Increased AChE activity induces rapid acetylcholine degradation and a resultant down stimulation of acetylcholine receptors, resulting in a decrease of cholinergic neurotransmission (Prasad, Muralidhara, 2012) which can explain the impairment in locomotor activity of flies exposed to ACR. ACR+4-PSQ significantly decreased AChE activity when compared to the ACR group. Similar results were observed by Couto et al. (2019) which 4-PSQ decreased the AChE activity to values close to the control group in *D. melanogaster* co-exposed with rotenone and 4-PSQ and Pinz et al. (2018) which 4-PSQ treatment prevented the increase of AChE

activity in the cerebral structures caused by amyloid β-peptide (Aβ) in a mouse model of Alzheimer's disease. One hypothesis proposed to explain the effect in the AChE activity is that, similar to other selenium-containing quinolone derivatives, 4-PSQ could be acting as a cholinesterase inhibitor (Pinz et al., 2018; Couto et al., 2019) suggesting that 4-PSQ may play a role in regulating cholinergic functions in the flies' heads. However, more studies are needed to explain the mechanisms that 4-PSQ has to restore the AChE activity.

In the present study, exposure to ACR significantly increased RS generation, and the ACR+4-PSQ attenuated RS generation. Many studies have already demonstrated that ACR neurotoxicity is associated with RS generation (Farouk et al., 2021, Sui et al., 2020, Zamani et al., 2017) and selenium compounds have been demonstrated to be strong oxygen radical scavengers (Ghorbel et al., 2017, Hafiz et al., 2020, Patra et al., 2018). Similar to previous studies where 4-PSQ had an antioxidant activity in *D. melanogaster* exposed to rotenone (Couto et al., 2019), tissues of aged rats (Luchese et al., 2020), atopic dermatitis-like skin lesions in mice (Voss et al., 2018), our results suggest a significant role of 4-PSQ in protecting the *D. melanogaster* against the RS generation produced by ACR exposure. Furthermore, we can observe a correlation between RS and MDA results. ACR exposure significantly increased MDA levels in *D. melanogaster* heads. MDA is a used marker of oxidative lipid injury and is produced as a result of RS-induced peroxidation of membrane lipids, which causes membrane damage and destruction (Li et al., 2017, Kong et al., 2016). ACR+4-PSQ showed a significant decrease in MDA levels which may be attributed to the ability of 4-PSQ to scavenge RS.

The antioxidant defense system includes antioxidant enzymes such as SOD and CAT and plays critical roles in cellular redox homeostasis protecting against intracellular oxidative stress (Reshmitha, Nisha, 2021). It is well established that ACR causes oxidative stress and, as a result, unbalances the cellular antioxidant homeostasis (Krishnan, Kang, 2019). Our studies demonstrated that treatment of flies with ACR exposed for 4 days suppressed the activity of SOD and caused the increase in the activity of CAT when compared to the control group. The reduction of SOD activity in ACR treated samples is related to the depletion of SOD enzymes used to neutralize RS generation caused by ACR and the increase of CAT activity. The increase of CAT activity in ACR-treated flies' heads can be explained by the probable inhibition of glutathione peroxidase. In *Drosophila*, when the reserves of glutathione peroxidase finish, CAT is the only enzyme that can eliminate hydrogen peroxide (Mockett et al., 2003). The co-

exposure of 4-PSQ significantly attenuated the changes of SOD and CAT activities by enhancing the antioxidant defense system. Similar results were observed in the liver, kidney, spleen, and cerebral cortex of aged rats where the CAT activity was restored after the treatment with 4-PSQ (Luchese et al., 2020). 4-PSQ also restored the activity of CAT and SOD in the head of flies exposed to rotenone (Couto et al., 2019) and in the body of flies exposed to a high sucrose diet (Silva et al., 2021).

GST enzymes are a large family of detoxifying enzymes that are involved in cellular detoxification and excretion of various xenobiotic molecules and it is known that high levels of GST activity are found in cytotoxic conditions (Allocati et al., 2018, Dasari et al. 2018a). In the main pathway of ACR elimination, ACR is conjugated with GSH to form mercapturic acid through GST (Uthra, et al., 2017). ACR exposed flies exhibited a GST increase in the head region as an attempt to detoxify by these enzymes. Similar results were found by Dasari et al. (2018b) in the brain of chicken embryo exposed to ACR. The co-exposure of ACR+4-PSQ significantly decreased GST activity indicating that 4-PSQ is capable of neutralizing the ACR neurotoxic effects.

In our study, ACR administration decreased cell viability in the flies' heads of MTT and Resazurin assay and ACR+4-PSQ increased the cell viability showing a protective effect against the loss of cell viability in the *D. melanogaster* heads. The decrease of cell viability in ACR-induced flies can be due to a variety of factors including impaired oxidative stress balance in the cells (Reshmitha; Nisha, 2021). It is known that selenium has protective effects against cellular death (Shalihat et al, 2021). The increasement of cell viability close to the control group observed in the co-exposure of 4-PSQ may be related to this antioxidant properties.

5. Conclusion

This study reports for the first time that 4-PSQ has a protective effect against ACR induced neurotoxicity in *D. melanogaster* through a mechanism that involves its regulation of AChE activity and consequent prevention of locomotor impairments. 4-PSQ is also associated to a protection against RS generation increasing the survival rate, MDA levels, the activity of CAT, SOD and GST and the cell viability in the flies' heads. Thus, our present study provides the potential application of 4-PSQ as a therapeutic treatment against ACR neurotoxicity.

Acknowledgments

The authors acknowledge the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their financial support.

References

Aebi, H., 1984. Catalase *in vitro*. Methods Enzymol. 105, 121-126. https://doi.org/10.1016/s0076-6879(84)05016-3.

Allocati, N., Masulli, M., Di Ilio, C., Federici, L., 2018. Glutathione transferases: substrates, inihibitors and pro-drugs in cancer and neurodegenerative diseases. Oncogenesis. 7, 1-15. https://doi.org/10.1038/s41389-017-0025-3.

Araujo, S., Paula, M., Poetini, M., Meichtry, L., Bortolotto, V., Zarzecki, M., Jesse, C., Prigol, M., 2015. Effectiveness of γ-orizanol in reducing neuromotor deficits, dopamine depletion and oxidative stress in a *Drosophila melanogaster* model of Parkinson's disease induced by rotenone. Neurotoxicology. 51, 96-105. https://doi.org/10.1016/j.neuro.2015.09.003.

Aydin, B., 2017. Effects of argan oil on the mitochondrial function, antioxidant system and the activity of NADPH- generating enzymes in acrylamide treated rat brain. Biomed Pharmacother. 87, 476-481. https://doi.org/476-481. 10.1016/j.biopha.2016.12.124.

Barth, A., Vogt, A., Reis, A., Pinz, M., Krüger, R., Domingues, W., Alves, D., Campos, V., Pinton, S., Paroul, N., Wilhelm, E. Luchese, C., 2019. 7-Chloro-4-(Phenylselanyl) Quinoline with memory enhancer action in aging rats: modulation of neuroplasticity, acetylcholinesterase activity, and cholesterol levels. Mol Neurobiol. 56, 1-11. https://doi.org/10.1007/s12035-019-1530-5.

Bolus, H., Crocker, K., Boekhoff-Falk, G., Chtarbanova, S. 2020. Modelling neurodegenerative disorders in *Drosophila melanogaster*. Int J Mol Sci. 9, 1-28. https://doi.org/10.3390/ijms21093055.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 7, 248-254. https://doi.org/10.1006/abio.1976.9999.

Chang, Y., Zeng, X., Sung, W., 2020. Effect of chitooligosaccharide and different low molecular weight chitosans on the formation of acrylamide and 5-hydroxymethylfurfural and Maillard reaction products in glucose/fructose-asparagine model systems. LWT-Food Sci Technol. 119, 1-7. https://doi.org/10.1016/j.lwt.2019.108879.

Chen, W., Su, H., Xu, Y., Jin, C. 2017. *In vitro* gastrointestinal digestion promotes the protective effect of blackberry extract against acrylamide-induced oxidative stress. Sci Rep. 7, 1-11. htps://doi.org/10.1038/srep40514.

Cheng, T., Yu, L., Ong, C., Bay, B., Baeg, G., 2019. The use of *Drosophila melanogaster* as a model organism to study immune-nanotoxicity. Nanotoxicology. 13, 429-446. https://doi.org/ 10.1080/17435390.2018.1546413.

Costa, G., Freita, C., Mendes, F., Roviero, J., Mutton, M., 2018. Acrylamide replaced by moringa extract in sugar production. Food Sci Technol. 38, 591-599. https://doi.org/ 10.1590/fst.00417.

Couto, S., Araujo, S., Bortolotto, V., Poetini, M, Pinheiro, F., Musachio, E., Meichtry, L., Sacramento, M., Alves, D., Novo, D., Mesko, M., Prigol, M., 2019. 7-chloro-4-(phenylselenyl) quinoline prevents dopamine depletion in a *Drosophila melanogaster* model of Parkinson's-like disease. J Trace Elem Med Biol. 54, 232-243, https://doi.org/ 10.1016/j.jtemb.2018.10.015.

Dasari, S., Ganajayi, M., Meriga, B. 2018a. Glutathione S-transferase is a good biomarker in acrylamide induced neurotoxicity and genotoxicity. Interdiscip Toxicol. 11, 115-121. https://doi.org/ 10.2478/intox-2018-0007.

Dasari, S., Gonuguntla, S., Meriga, B., Kedam, T. 2018b. Determination of acrylamide-induced chick embryo brain glutathione S-transferases expression through enzyme activity and western blot. J Applied Biol Biotechnol. 61, 43-47. https://doi.org/ 10.7324/JABB.2018.60108.

Dominiak, A., Wilkaniec, A., Jęśko, H., Czapski, G., Lenkiewicz, A., Kurek, E., Wroczyński, P., Adamczyk, A., 2017. Selol, an organic selenium donor, prevents lipopolysaccharide-induced oxidative stress and inflammatory reaction in the rat brain. Neurochem Int. 108, 66-77. https://doi.org/10.1016/j.neuint.2017.02.014.

Duan, X., Wang, Q., Chen, K., Zhu, C., Liu, J., Sun, S., 2015. Acrylamide toxic effects on mouse oocyte quality and fertility *in vivo*. Sci Rep. 5, 1-11. https://doi.org/ 10.1038/srep11562.

Elblehi, S., Euony, O., El-Sayed, Y., 2020. Apoptosis and astrogliosis perturbations and expression of regulatory inflammatory factors and neurotransmitters in acrylamide-induced neurotoxicity under ω3 fatty acids protection in rats. Neurotoxicology. 76, 44-57. https://doi.org/ 10.1016/j.neuro.2019.10.004.

Ellman, G., Courtney, K., Andres, V., Featherstone, R., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 7, 88-95. https://doi.org/0.1016/0006-2952(61)90145-9.

Faria, M., Prats, E., Canela, C., Hsu, C., Arick, M., Bedrossiantz, J., Orozco, M., Reyero, N., Ziv, T., Lulu, S., Admon, A., Oliván, L., Raldúa, D., 2019. Therapeutic potential of N-acetylcysteine in acrylamide acute neurotoxicity in adult zebrafish. Sci Rep. 9, 1-11. https://doi.org/ 10.1038/s41598-019-53154-w.

Farouk, S., Gad, F., Almeer, R., Daim, M., 2021. Exploring the possible neuroprotective and antioxidant potency of lycopene against acrylamide-induced neurotoxicity in rats' brain. Biomed Pharmacother. 138, 1-12. https://doi.org/10.1016/j.biopha.2021.111458.

Franco, J., Posser, T., Dunkley, P., Dickson, P., Mattos, J., Martins, R., Bainy, A., Marques, M., Dafre, A., Farina, M., 2009. Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. Free Radic biol Med. 4, 449-457. https://doi.org/10.1016/j.freeradbiomed.2009.05.013.

Ghorbel, I., Elwej, A., Chaabane, M., Jamoussi, K., Mnif, H. Boudawara, T., Zeghal, N., 2017. Selenium alleviates oxidative stress and lung damage induced by aluminum chloride in adult rats: biochemical and histological approach. Biol Trace Elem Res. 176, 181-191. https://doi.org/10.1007/s12011-016-0818-9.

Habig, W., Pabst, M., Jakoby, W., 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem. 22, 7130-7139. PMID: 4436300.

Hafiz, F., El-Sayed, N., Khodeer, D., Ahmed, A., Hanna, P., Moustafa, Y., 2020. Nano selenium ameliorates oxidative stress and inflammatory response associated with cypermethrin-induced neurotoxicity in rats. Ecotoxicol Environ Saf. 195, 1-7. https://doi.org/10.1016/j.ecoenv.2020.110479.

Hirth, F., 2010. *Drosophila melanogaster* in the study of human degeneration. CNS Neurol Disord Drug Targets. 4, 504-524. https://doi.org/10.2174/187152710791556104.

Hosamani, R., Ramesh, S., Mudalidhara., 2010. Attenuation of rotenone-induced mitochondrial oxidative damage and neurotoxicity in *Drosophila melanogaster* supplemented with creatine. Neurochem Res. 35, 1402-1412. https://doi.org/10.1007/s11064-010-0198-z.

Huang, M., Jiao, J., Wang, J., Xia, Z., Zhang, Y., 2018. Characterization of acrylamide-induced oxidative stress and cardiovascular toxicity in zebrafish embryos. J Hazard Mater. 347, 451-460. https://doi.org/10.1016/j.jhazmat.2018.01.016.

Hussaini, S. 2016. Therapeutic significance of quinolines: a patent review (2013-2015). Expert Opin Ther Pat. 10, 1201-1221. https:// doi.ogrg/10.1080/13543776.2016.1216545.

Kong, W., Liu, F., Zhang, C., Zhang, j., Feng, H., 2016. Non-destructive determination of Malondialdehyde (MDA) distribution in oilseed rape leaves by laboratory scale NIR hyperspectral imaging. Sci Rep. 6, 1-8. https://doi.org/10.1038/srep35393.

Kostyuk, V., Potapovich, A., 1989. Superoxide-driven oxidation of quercetin and a simple sensitive assay for determination of superoxide dismutase. Biochem Int. 5, 1117-1124. PMID: 2561443.

Krishnan, M., Kang, S. 2019. Vitexin inhibits acrylamide-induced neuroinflammation and improves behavioral changes in zebrafish larvae. Neurotoxicol Teratol. 74, 1-10. https://doi.org/10.1016/j.ntt.2019.106811.

Li, L., Sun, H., Liu, W. Zhao, H., Shao, M., 2017. Silymarin protects against acrylamide-induced neurotoxicity via Nrf2 signalling in PC12 cells. Food Chem Toxicol. 102, 93-101. https://doi.org/10.1016/j.fct.2017.01.021.

Liu, Y., Yan, D., Wang, Y., Zhang, X., Wang, N., Jiao, Y., Yan, H., 2021. Subchronic exposure to acrylamide caused behaviour disorders and related pathological and molecular changes in rat cerebellum. Toxicol Lett. 340, 23-32. https://doi.org/ 10.1016/j.toxlet.2021.01.009.

Luchese, C., Barth, A., Costa, G., Alves, D., Novo, D., Mesko, M., Wilhelm, E., 2020. Role of 7-chloro-4-(phenylselanyl) quinoline as an anti-aging drug fighting oxidative damage in different tissues of aged rats. Exp Gerontol. 9. https://doi.org/10.1016/j.exger.2019.110804.

Matoso, V., Souza, P., Ivanski, F., Romano, M., Romano, R., 2019. Acrylamide: a review about its toxic effects in the light of developmental origin of health and disease (DOHaD) concept. Food Chem. 283, 422-430. https://doi.org/10.1016/j.foodchem.2019.01.054.

Mockett, R., Baybe, A., Kwong, L., Orr, W., Sohal, R. 2003. Ectopic expression of catalase in *Drosophila* mitochondria increases stress resistance but not longevity. Free Radic Biol Med. 34, 207-217. https://doi.org/10.1016/s0891-5849(02)01190-5.

Mottram, D., Wedzicha, B., Dodson, A. 2002. Food chemistry: acrylamide is formed in the Maillard reaction. Nature, 419, 448-449. https://doi.org/10.1038/419448a.

Murray, S., Waddell, B., Wu, C. Neuron-specific toxicity of chronic acrylamide exposure in *C. elegans*. Neurotoxicol Teratol. 77, 1-10. https://doi.org/ 10.1016/j.ntt.2019.106848.

Nishihara, S., 2020. Functional analysis of glycosylation using *Drosophila melanogaster*. Glycoconj J. 37, 1-14. https://doi.org/10.1007/s10719-019-09892-0.

Nomi, Y., Otsuka, Y., 2020. Isolation, identification and proposed formation mechanism of a novel hydrophilic compound formed by Maillard reaction between pyridoxamine and pentose. Sci Rep. 10, 1-9. https://doi.org/10.1038/s41598-020-58727-8.

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 2, 351-358. https://doi.org/10.1016/0003-2697(79)90738-3.

Paltian, J., Reis, A., Oliveira, R., Fonseca, C., Domingues, W., Dellagostin, E., Campos, V., Kruger, R., Alves, D., Luchese, C., Wilhelm, E., 2020. The anxiolytic effect of a promising quinoline containing selenium with the contribution of the serotonergic and GABAergic pathways: Modulation of parameters associated with anxiety in mice. Behav Brain Res. 393, 1-10. https:// doi.org/10.1016/j.bbr.2020.112797.

Park, J., Samanta, P., Lee, S., Lee, J., Cho, J., Chun, H., Yoon, S., Kim, W., 2021. Developmental and neurotoxicity of acrylamide to zebrafish. Int J Mol Sci. 22, 1-12. https://doi.org/10.3390/ijms22073518.

Patra, A., Roy, S., Basu, A., Bhuniya, A., Bhattacharjee, A., Hajra, S., Hossain, U., Baral, R., Bhattacharya, S., 2018. Design and synthesis of coumarn-based organoselenium as a

new hit for myeloprotection and synergistic therapeutic efficacy in adjuvant therapy. Sci Rep. 8, 1-12. https://doi.org/10.1038/s41598-018-19854-5.

Perez-Severiano, F., Santamaría, A., Chaverri, J., Campos, O. Ríos, C., Segovia, J., 2004. Increased formation of reactive oxygen species, but no changes in glutathione peroxidase activity, in striata of mice transgenic for the Huntington's disease mutation. Neurochem Res. 4, 729-733. https://doi.org/10.1023/b:nere.0000018843.83770.4b.

Pinz, M., Reis, A., Vogt, A., Krüger, Alves, D., Jesse, C., Roman, S., Soares, M., Wilhelm, E., Luchese, C., 2018. Current advances of pharmacological properties of 7-chloro-4-(phenylselanyl) quinoline: Prevention of cognitive deficit and anxiety in Alzheimer's disease model. Biomed Pharmacother. 105, 1006- 1014. https://doi.org/10.1016/j.biopha.2018.06.049.

Prasad, S., Muralidhara., 2012. Evidence of acrylamide induced oxidative stress and neurotoxicity in *Drosophila melanogaster* – Its amelioration with spice active enrichment: Relevance to neuropathy. Neurotoxicology. 33, 1254-1264. https://doi.org/10.1016/j.neuro.2012.07.006.

Prasad, S., Muralidhara., 2014. Neuroprotective effect of geraniol and curcumin in an acrylamide model of neurotoxicity in *Drosophila melanogaster*: relevance to neuropathy. J Insect Physiol. 60, 7-16. https://doi.org/10.1016/j.jinsphys.2013.10.003.

Reis, A., Paltian, J., Domingues, W., Costa, G., Giongo, J., Campos, V., Luchese, C., Wilhelm, E., 2020. Pharmacological modulation of Na+, K+-ATPase as a potential target for OXA-induced neurotoxicity: correlation between anxiety and cognitive decline and beneficial effects of 7-chloro-4-(phenylselanyl) quinoline. Brain Res Bull. 162, 282-290. https:// 10.1016/j.brainresbull.2020.06.021.

Reshmitha, T., Nisha, P., 2021. Lycopene mitigates acrylamide and glycidamide induced cellular toxicity via oxidative stress modulation in HepG2 cells. J Funct Foods. 80, 1-8. https://doi.org/10.1016/j.jff.2021.104390.

Sansano, M., Heredia, A., Peinado, I., Andrés, A., 2017. Dietary acrylamide: What happens during digestion. Food Chem. 237, 58-64. https://doi.org/10.1016/j.foodchem.2017.05.104.

Savegnago, L., Vieira, A., Seus, N., Goldani, B., Castro, M., Lenardão, E., Alves, D., 2013. Synthesis and antioxidant properties of novel quinoline-chalcogenium compounds. Tetrahedron Lett. 54, 40-44. https://doi.org/10.1016/j.cbpc.2020.108892.

Senthilkumar, S., Raveendran, R., Madhusoodanan, S., Sundar, M., Shankar, S., Sharma, S., Sundarajan, V., Dan, P., Mohideen, S., 2020. Developmental and behavioural toxicity induced by acrylamide exposure and amelioration using phytochemicals in *Drosophila melanogaster*. J Hazard Mater. 394, 1-10. https://doi.org/10.3390/ijms21093055.

Shakya, B., Siddique, Y., 2018. Exploring the neurotoxicity and changes in life cycle parameters of *Drosophila melanogaster* exposed to arecoline. JoBAZ. 79, 1-11. https://doi.org/10.1186/s41936-018-0057-z.

Shalihat, A., Hasanah, A., Mutakin, Lesmana, R., Budiman, A., Gozali, D. The role of selenium in cell survival and its correlation with protective effects against cardiovascular disease: a literature review. Biomed Pharmacother. 134, 1-8. https://doi.org/ 10.1016/j.biopha.2020.111125.

Silva, D., Silva, M., Guerra, G., Sacramento, M., Alves, D., Prigol, M., 2021. 7-chloro-4-(phenylselanyl) quinoline co-treatment prevent oxidative stress in diabetic-like phenotype induced by hyperglycidic diet in *Drosophila melanogaster*. Comp Biochem Physiol C Toxicol Pharmacol. 239, 1-10. https://doi.org/10.1016/s0891-5849(02)01190-10.1016/j.cbpc.2020.108892.

Stadler, R., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P., Robert, M., Riediker, S., 2002. Acrylamide from Maillard reaction products. Nature, 419, 449-450. https://doi.org/10.1038/419449a.

Sui, X., Yang, J., Zhang, G., Yuan, X., Li, W., Long, J., Luo, Y., Li, Y., Wang, Y., 2020. NLRP3 inflammasome inhibition attenuates subacute neurotoxicity induced by acrylamide *in vitro* and *in vivo*. Toxicology. 432, 1-13. https://10.1016/j.tox.2020.152392.

Uthra, C., Shrivastava, S., Jaswal, A., Sinha, N., Reshi, M., Shukla, S., 2017. Therapeutic potential of quercetin against acrylamide induced toxicity in rats. Biomed Pharmacother. 86, 705-714. https://doi.org/ 10.1016/j.biopha.2016.12.065.

Vogt, A., Voss, G., Oliveira, R., Paltian, J., Duarte, L., Alves, D., Jesse, C., Roman, S., Roehrs, J., Wilhelm, E., Luchese, C., 2018. Organoselenium group is critical for antioxidant activity of 7-chloro-4-phenylselenyl-quinoline. Chem Biol Interact. 282, 7-12. https://doi.org/10.1016/j.cbi.2018.01.003.

Voss, G., Oliveira, R., Souza, J., Duarte, L., Fajardo, A., Alves, D., Luchese, C., Wilhelm, E., 2018. Therapeutic and technological potential of 7-chloro-4-phenylselanyl quinoline for the treatment of atopic dermatitis-like skin lesions in mice. Mater Sci Eng C mater Biol Appl. 84, 90-98. https://doi.org/10.1016/j.msec.2017.11.026.

Zamani, E., Shokrzadeh, M., Fallah, M., Shaki, F., 2017. A review of acrylamide toxicity and its mechanism. Pharm Biomed Res. 3, 1-7. https://doi.org/10.18869/acadpub.pbr.3.1.1.

Zhao, M., Wang, F., Hu, X., Chen, F., Chan, H., 2017. Effect of acrylamide-induced neurotoxicity in primary astrocytes/microglial co-culture model. Toxicol *in vitro*. 39, 119-125. https://doi.org/10.1016/j.tiv.2016.11.007.

Figures:

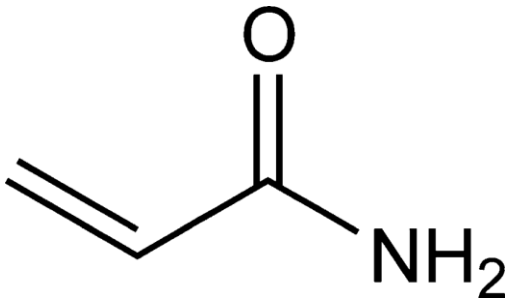


Figure 1. Chemical structure of Acrylamide (ACR).

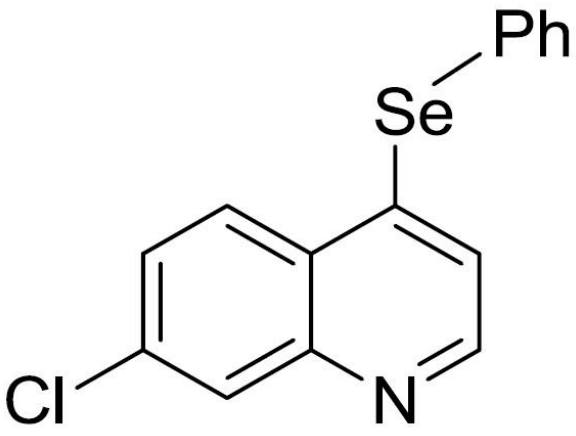


Figure 2. Chemical structure of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ).

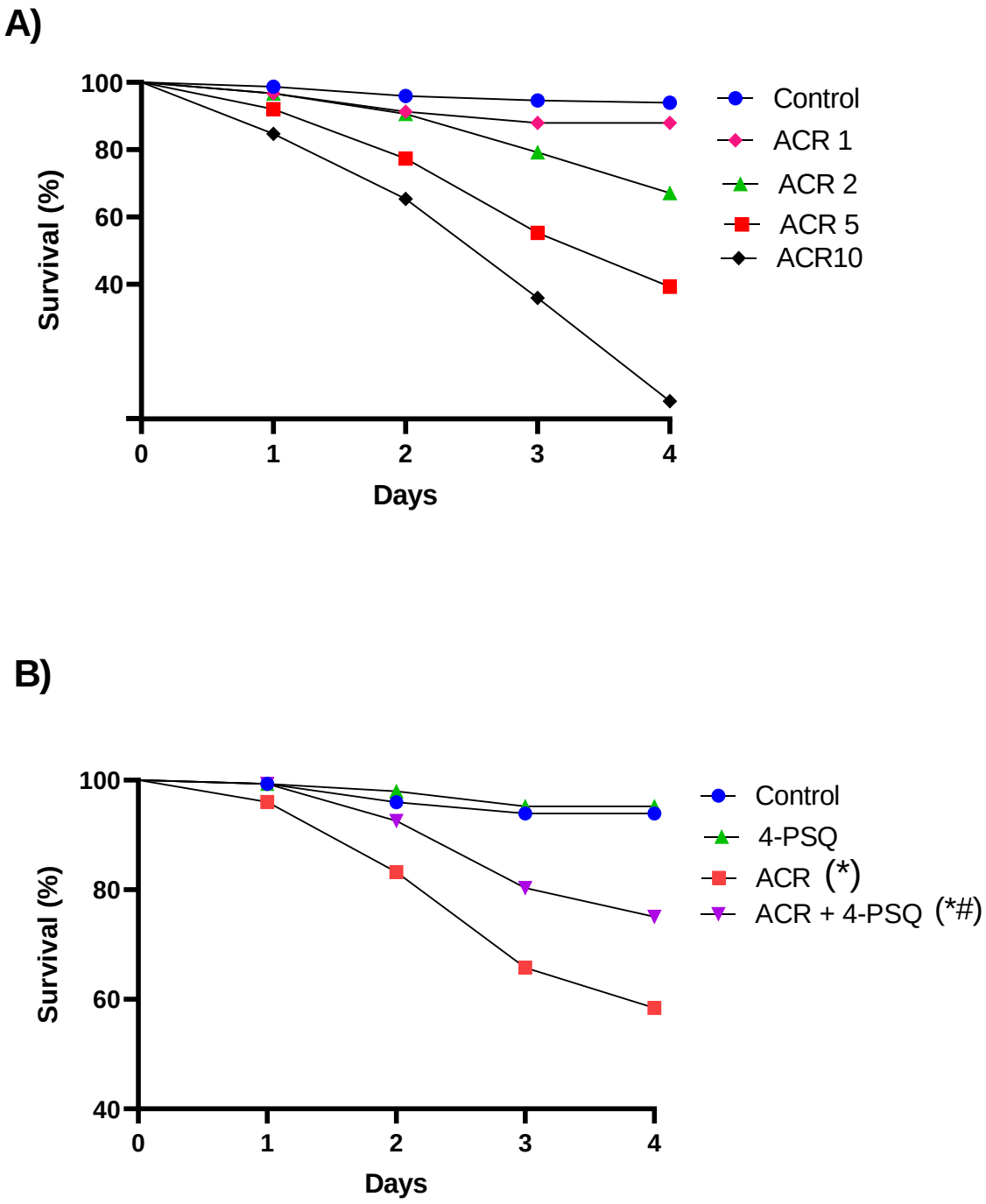


Figure 3. (A) Effect of ACR exposure (4 days) on survival rate. (B) Effects of 4-PSQ treatment to ACR exposed flies on survival rate. Data were collected every 24 h for each group during 4 days. The total number of flies represents the sum of three independents experiments. Lifespan measurement was determined by comparing the survival curves Mantel-Cox log-rank test and multiple comparisons were corrected using the Bonferroni method. *Significant difference compared to the control group ($p < 0.05$).

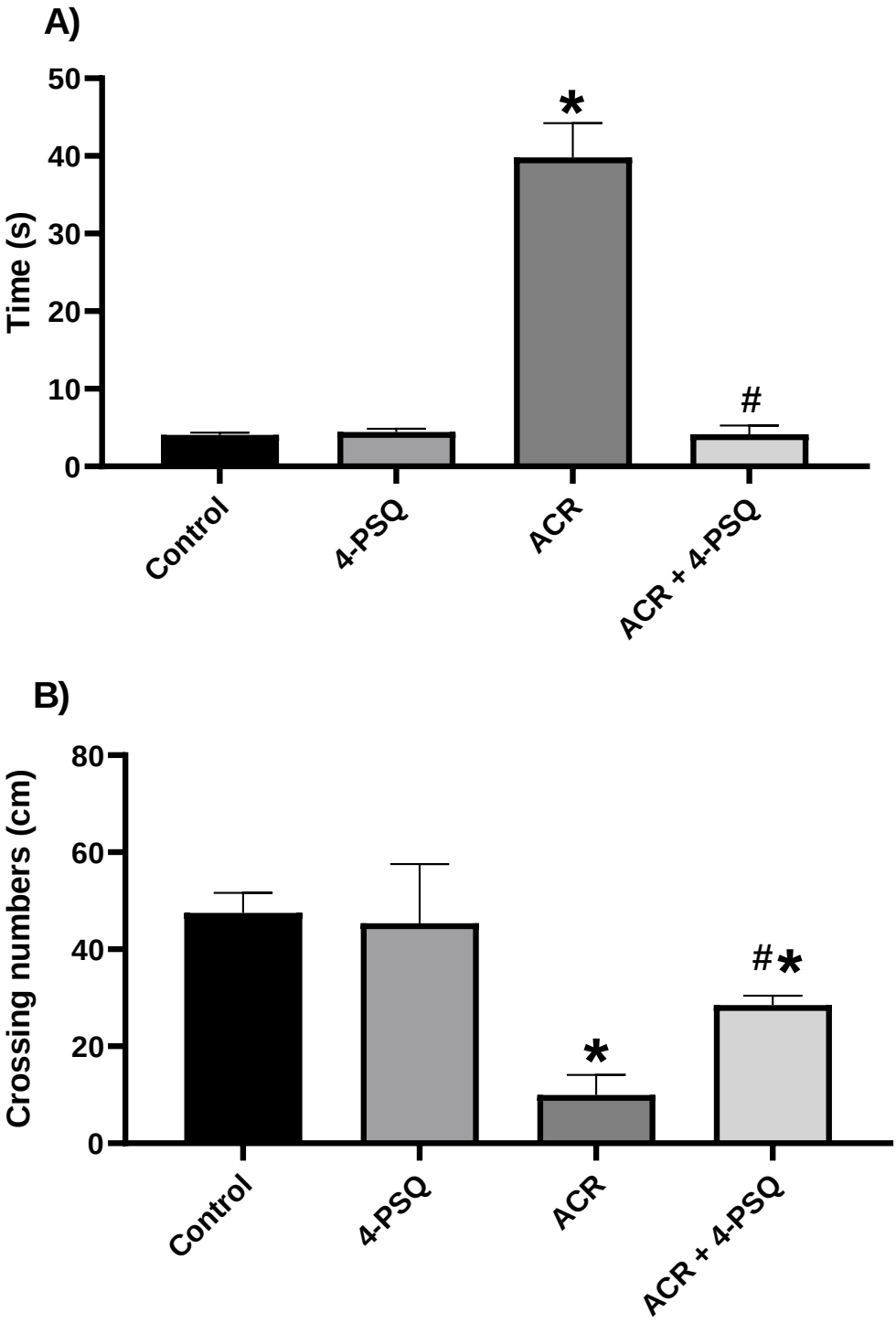


Figure 4. Effects of 4-PSQ treatment to ACR exposed *D. melanogaster* on geotaxic response (climbing) and exploratory activity of flies exposed during 4 days. (A) Negative geotaxis test, (B) Open field assay. Values are mean ± SE. Significance determined by One-way analysis of variance (ANOVA) followed by Bonferroni test. *Significant

difference compared to the control group ($p<0.05$); #Significant difference between ACR and ACR+4-PSQ ($p<0.05$).

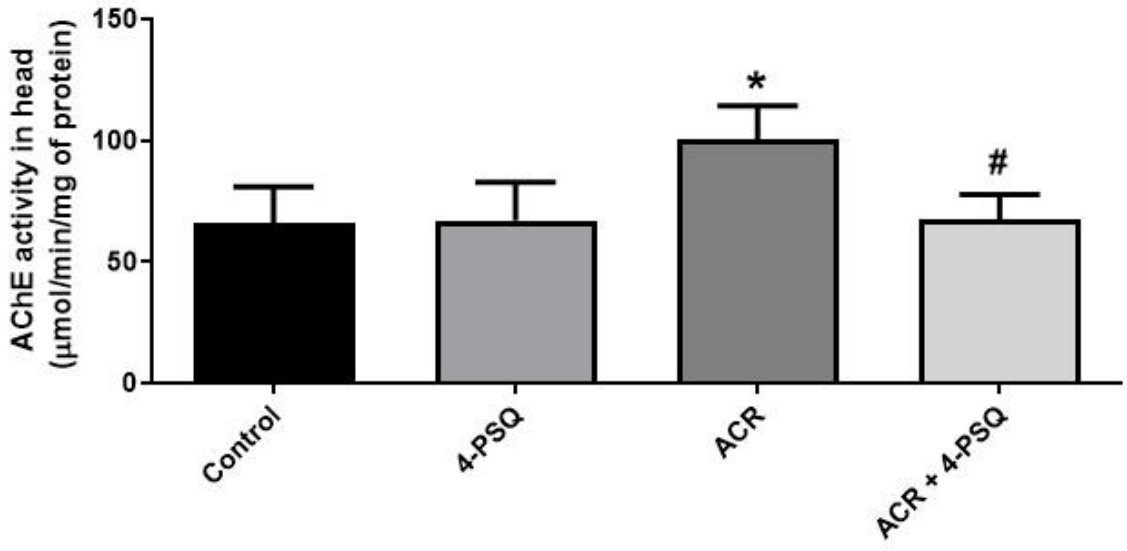


Fig. 5 Effects of 4-PSQ treatment to ACR exposed flies in *D. melanogaster* heads on levels of acetyl cholinesterase (AChE). Values are mean ± SE. Significance determined by One-way analysis of variance (ANOVA) followed by Bonferroni test. *Significant difference compared to the control group ($p<0.05$); #Significant difference between ACR and ACR+4-PSQ ($p<0.05$).

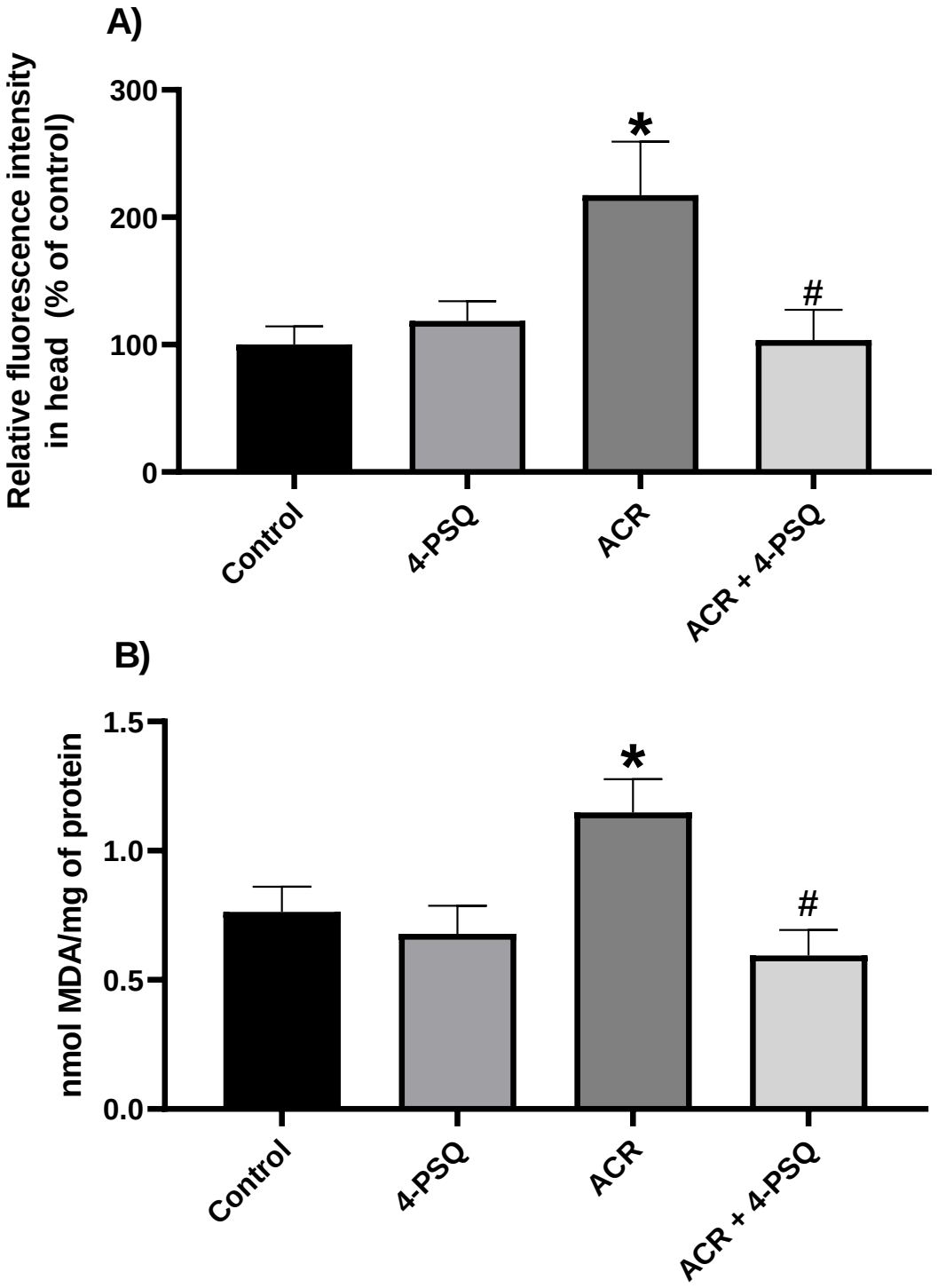


Fig. 6 Effects of 4-PSQ treatment to ACR exposed flies in *D. melanogaster* heads on levels of oxidative stress markers. (A) RS (B) MDA. Values are mean ± SE. Significance determined by One-way analysis of variance (ANOVA) followed by Bonferroni test. *Significant difference compared to the control group ($p<0.05$); #Significant difference between ACR and ACR+4-PSQ ($p<0.05$).

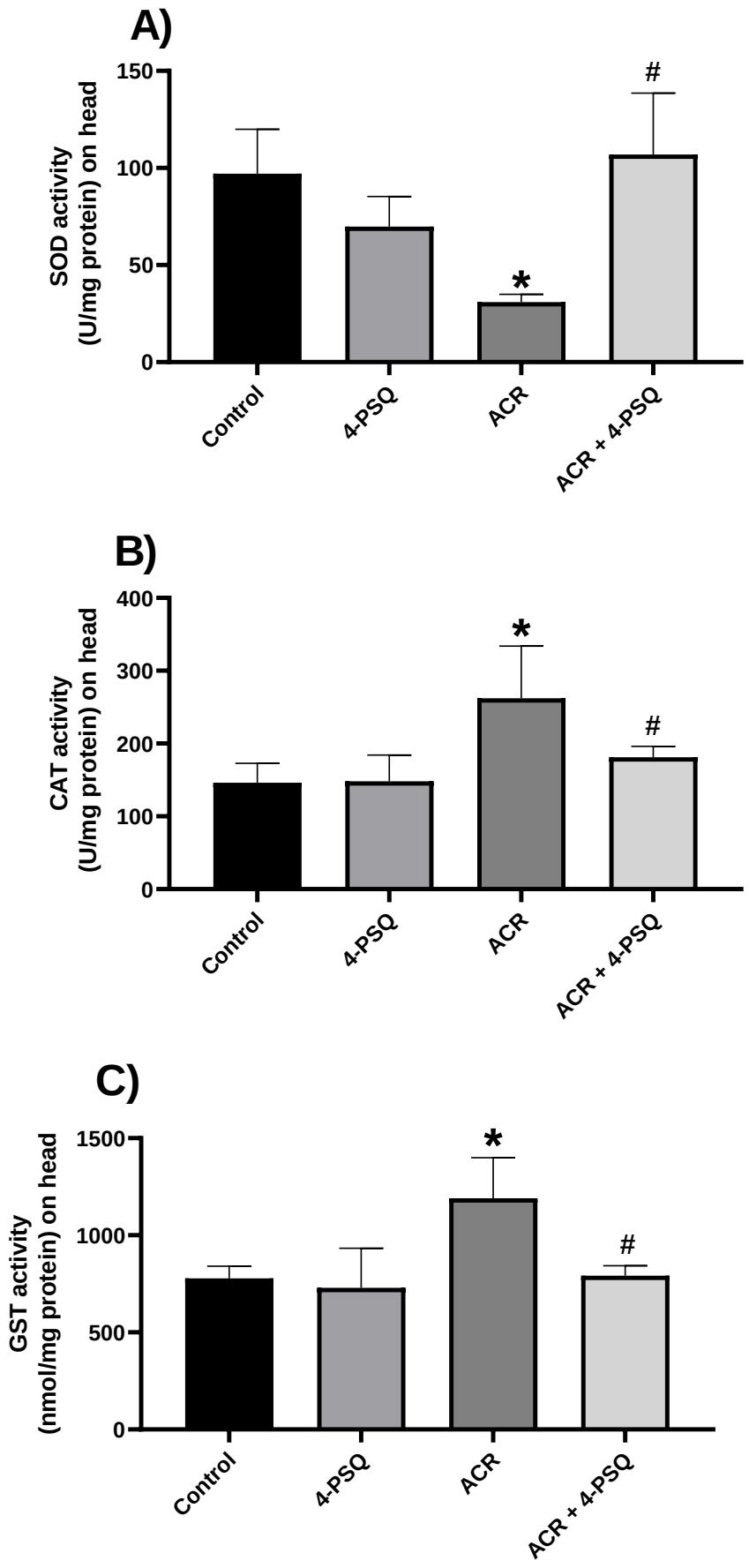

A)
SOD activity
(U/mg protein) on head
150
100
50
0
*
#
Control
4-PSQ
ACR
ACR + 4-PSQ
B)
CAT activity
(U/mg protein) on head
400
300
200
100
0
*
#
Control
4-PSQ
ACR
ACR + 4-PSQ
C)
GST activity
(nmol/mg protein) on head
1500
1000
500
0
*
#
Control
4-PSQ
ACR
ACR + 4-PSQ

Fig. 7 Effects of 4-PSQ treatment to ACR exposed flies in *D. melanogaster* heads on levels of antioxidant enzymes. (A) SOD (B) CAT (C) GST. Values are mean ± SE. Significance determined by One-way analysis of variance (ANOVA) followed by Bonferroni test. *Significant difference compared to the control group ($p<0.05$); #Significant difference between ACR and ACR+4-PSQ ($p<0.05$).

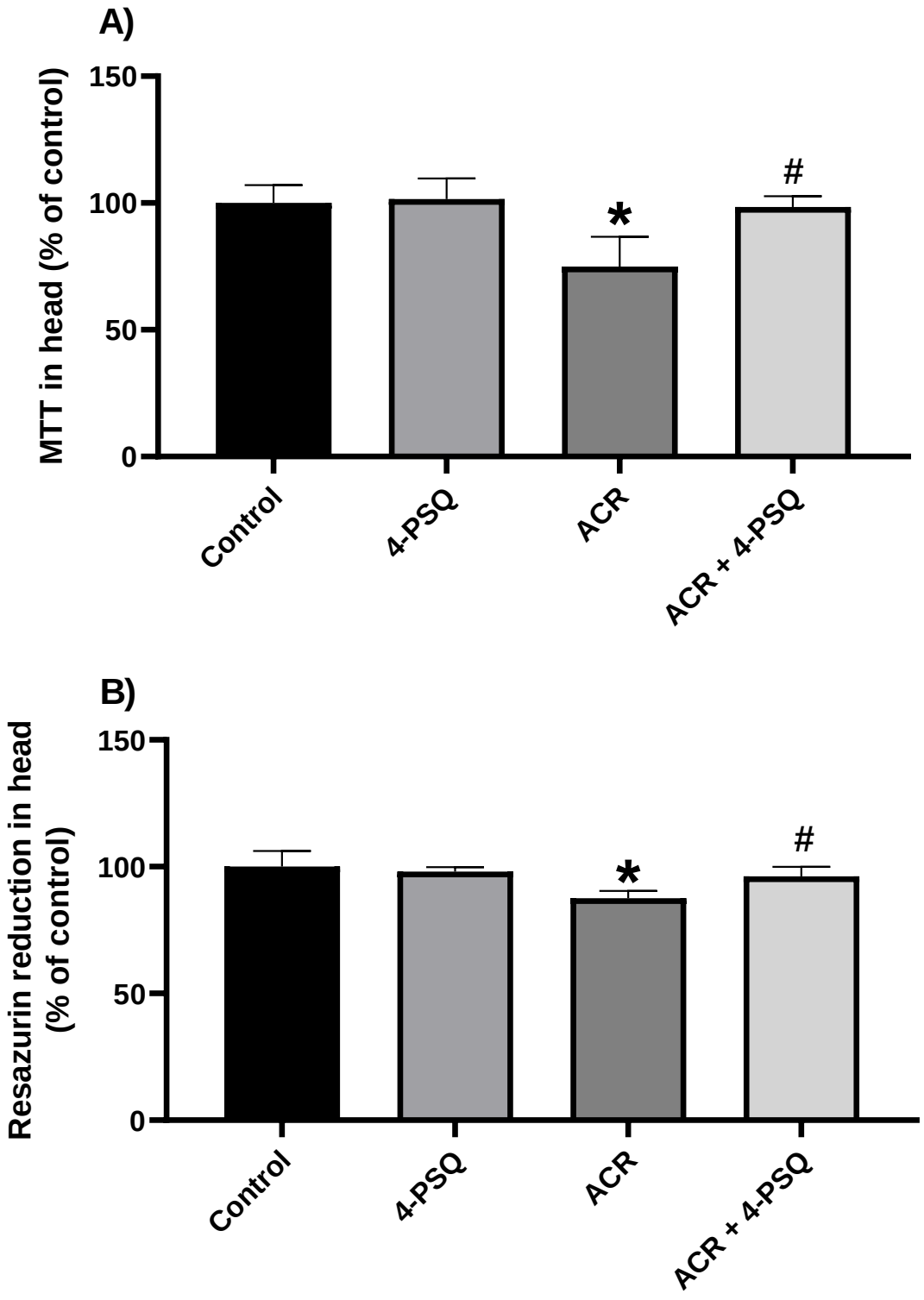


Fig. 8 Effects of 4-PSQ treatment to ACR exposed flies in *D. melanogaster* heads on the levels of cell viability markers. (A) MTT (B) Resazurin. Values are mean ± SE. Significance determined by One-way analysis of variance (ANOVA) followed by

Bonferroni test. *Significant difference compared to the control group ($p<0.05$); #Significant difference between ACR and ACR+4-PSQ ($p<0.05$).

6. CONCLUSÕES

Com base nos resultados obtidos no presente trabalho, conclui-se que o 4-PSQ foi capaz de restaurar os danos locomotores causados pela ACR através de sua ação de regulação da atividade da AChE. O 4-PSQ mostrou-se ainda, um composto com propriedade antioxidante, aumentando as taxas de sobrevivência e restaurando os níveis de MDA, as atividades das enzimas CAT, SOD e GST e a viabilidade celular na cabeça das moscas. Diante dos nossos dados, demonstra-se que o 4-PSQ apresenta-se como um potencial alvo terapêutico contra a neurotoxicidade induzida pela exposição das *D. melanogaster* à ACR.

7. PERSPECTIVAS FUTURAS

A busca por novos compostos com propriedades antioxidantes é de extrema importância tendo em vista que a maioria das patologias possui algum tipo de ligação com o estresse oxidativo. Diante dos resultados positivos encontrados em nosso trabalho, é necessária uma pesquisa mais profunda deste composto, a fim de ampliar o conhecimento de seus mecanismos de ação e possíveis interações. É importante ainda, realizar mais estudos sobre o efeito anticolinesterásico do composto tendo em vista seu potencial uso em tratamentos farmacológicos. O prosseguimento deste trabalho tornará nossa pesquisa mais completa e elucidará prováveis lacunas ainda não estudadas na pesquisa com este composto de uma forma geral.

REFERÊNCIAS

ANDERSEN, F. A. Amended final report on the safety assessment of polyacrylamide and acrylamide residues in cosmetics. **International Journal of Toxicology**, v. 24, p. 21-50, 2005.

BARTH, et al. 7-Chloro-4-(Phenylselanyl) Quinoline with memory enhancer action in aging rats: modulation of neuroplasticity, Acetylcholinesterase Activity, and Cholesterol Levels. **Molecular Neurobiology**, v. 56, n. 9, p. 1-11, 2019.

BERTUZZI, T. et al. Acrylamide determination during an industrial roasting process of coffee and the influence of asparagine and low molecular weight sugars. **Food Chemistry**, v. 303, p.1-6, 2020.

BOLUS, H. et al. Modelling neurodegenerative disorders in *Drosophila melanogaster*. **International Journal of Molecular Sciences**, p.1-28, 2020.

CHENG, T. et al. The use of *Drosophila melanogaster* as a model organism to study immune-nanotoxicity. **Nanotoxicology**, v. 13, n. 4, p.429-446, 2019.

COSTA et al. Acrylamide replaced by moringa extract in sugar production. **Food Science and Technology**, v. 38, n. 4, p. 591-599, 2018.

COUTO, S. F. et al. 7-chloro-4-(phenylselenyl) quinoline prevents dopamine depletion in a *Drosophila melanogaster* model of Parkinson's-like disease. **Journal of Trace Elements in Medicine and Biology**, 2019.

DE MEULENAER, B.; MEDEIROS, R.; MESTDAGH, F. **Acrylamide in potato products.** In: Advances in potato chemistry and technology. 2. ed. 527-562, 2016. 752 p.

DEMIR, E. *Drosophila* as a model for assessing nanopesticide toxicity. **Nanotoxicology**, v. 14, p. 1271-1279, 2020.

DOMINIAK, A. et al. Selol, an organic selenium donor, prevents lipopolysaccharide-induced oxidative stress and inflammatory reaction in the rat brain. **Neurochemistry International**, v. 108, p. 66-77, 2017.

ELBLEHI, S.; EYINY, O.; EL-SAYED, Y. Apoptosis and astrogliosis perturbations and expression of regulatory inflammatory factors and neurotransmitters in acrylamide-induced neurotoxicity under ω3 fatty acids protection in rats. **NeuroToxicology**, v. 76, p. 44-57, 2020.

FARIA, M. et al. Therapeutic potential of N-acetylcysteine in acrylamide acute neurotoxicity in adult zebrafish. **Scientific Reports**, v. 9, p. 1-11, 2019.

FENNEL, T. et al. Kinectics of elimination of urinary metabolites of acrylamide in humans. **Toxicological Sciences**, v. 93, n. 2, p. 256-267, 2006.

GHORBEL, I. et al. Selenium alleviates oxidative stress and lung damage induced by aluminum chloride in adult rats: biochemical and histological approach. Biological Trace **Element Research**, v. 176, p. 181-191, 2017.

HALFORD, N.; RAFFAN, S. **Acrylamide: new European risk management measures and prospecs for reducing the acrylamide-forming potenial of wheat.** In: GRANVOGL; MACMAHON. Food-Borne toxicants: Formation, analysis, and toxicology. ACS Symposium Series; American Chemical Society: Washington, DC, 2019.

HAMZAHOĞLU, A.; GÖKMEN, V. **Formation of acrylamide in thermally processed foods and its reactions during** *in vitro* **digestion**. In: GRANVOGL; MACMAHON. Food-Borne toxicants: Formation, analysis, and toxicology. ACS Symposium Series; American Chemical Society: Washington, DC, 2019.

HASCHEK, W.; ROUSSEAUX, C.; WALLIG, M. **Nervous System**. In: WALLIG, M.; HASCHEK, W.; ROUSSEAUX, C. Fundamentals of Toxicologic Pathology. Academic Press: Cambridge, Massachusetts, 2010.

HUANG, M. et al. Characterization of acrylamide-induced oxidative stress and cardiovascular toxicity in zebrafish embryos. **Journal of Hazardous Materials**, v. 347, p. 451-460, 2018.

HUSSAINI, S. Therapeutic significance of quinolines: a patent review (2013-2015). **Expert Opinion on Therapeutic Patents**, v. 10, p.1201-1221, 2016.

IARC. International Agency for Cancer Research. **Acrylamide**. In: IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans, v. 60, p. 389–433, 1994.

JENNINGS. B. *Drosophila* – a versatile model in biology & medicine. **Materials Today**, v. 14, n. 5, p. 190-195, 2011.

KATEN, A.; ROMAN, S. The genetic consequences of paternal acrylamide exposure and potential for amelioration. **Mutation Rechearch/ Fundamental and Molecular Mechanisms of Mutagenis**, v. 777, p. 91-100, 2015.

KOMOIKE, Y.; MATSUOKA, M. *In vitro* and *in vivo* studies of oxidative stress responses against toxicity in zebrafish. **Journal of Hazardous Materials**, v. 365, p. 430-439, 2019.

LOPACHIN, R. M.; GAVIN, T. Acrylamide-induced nerve terminal damage: relevance to neurotoxic and neurodegenerative mechanisms. **Journal of Agricultural and Food Chemistry,** v. 56, n..15, p. 5994-6006, 2008.

LOPACHIN, R. M.; GAVIN, T. Acrylamide and related α,β-unsaturated carbonyl derivatives. In: AMINOFF, M. J.; DAROFF, R.B. (org.). **Encyclopedia of the Neurological Sciences**. 2. ed. Cambridge: Academic Press, 2014, p.29-32.

LUCHESE, C. et al. Role of 7-chloro-4-(phenylselanyl) quinoline as an anti-aging drug fighting oxidative damage in different tissues of aged rats. **Experimental Gerontology**, 2020.

MATOSO, V. et al. Acrylamide: a review about its toxic effects in the light of developmental origin of health and disease (DOHaD) concept. **Food Chemistry**, v. 283, p.422-430, 2019.

MIRZOYAN, Z. *Drosophila melanogaster*. A model organism to study cancer. **Frontiers in Genetics**, v. 10, n. 51, p.1-16, 2019.

MOJSKA, H.; GLELECLŃSKA, I.; CENDROWSKL, A., Acrylamide content in cigarette mainstream smoke and estimation of exposure to acrylamide from tobacco smoke in Poland. **Annals of Agricultural and Environmental Medicine**, v. 23, n. 3, p. 456-461, 2016.

MOTTA, K. et al. 7-Chloro-4- (phenylselanyl) quinoline reduces renal oxidative stress induced by oxaliplatin in mice. **Canadian Journal of Physiology and Pharmacology**, v. 20, p. 1-10,2021.

MOTTRAM, D.; WEDZICHA, B.; DODSON, A. Food chemistry: acrylamide is formed in the Maillard reaction. **Nature**, v. 419, p. 448-449, 2002.

MURRAY, S.; WADDELL, B.; WU, C. Neuron-specific toxicity of chronic acrylamide exposure in *C. elegans*. **Neurotoxicology and Teratology**, v. 77, p. 1-10, 2020.

National Center for Biotechnology Information. PubChem Database. **Acrylamide, CID=6579**. Disponível em: <https://pubchem.ncbi.nlm.nih.gov/compound/Acrylamide>. Acesso em: 6 mar. 2020.

NISHIHARA, S. Functional analysis of glycosylation using *Drosophila melanogaster*. **Glycoconjugate Journal**, v. 37, p. 1-14, 2020.

ONG, C. et al. *Drosophila melanogaster* as a model organism to study nanotoxicity. **Nanotoxicology**, v. 9, n. 3, p. 1-8, 2015.

OU, J. et al. Interaction of acrylamide, acrolein, and 5-hydroxymethylfurfural with amino acids and DNA. **Journal of Agricultural and Food Chemistry**, v. 68, n. 18, p. 5030-5048, 2020.

PALTIAN, J. et al. The anxiolytic effect of a promising quinoline containing selenium with the contribution of the serotonergic and GABAergic pathways: Modulation of parameters associated with anxiety in mice. **Behavioural Brain Research**, v. 393, p.1-10, 2020.

PALUS, K.; CAŁKA, J. Influence of acrylamide administration on the neurochemical characteristics of enteric nervous system (ENS) neurons in the porcine duodenum. **International Journal of Molecular Sciences**, v. 21, n. 15, p.1-14, 2020.

PAN, X. et al. Acrylamide-induced oxidative stress and inflammatory response are alleviated by N-acetylcysteine in PC12 cells: Involvement of the crosstalk between Nrf2 and NF-κB. **Toxicology Letters**, v. 288, p.55-64, 2018.

PANDEY, U.; NICHOLS, C. Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. **Pharmacological Reviews**, v. 63, n. 2, p. 411-436, 2011.

PATRA et al. Design and synthesis of coumarin-based organoselenium as a new hit for myeloprotection and synergistic therapeutic efficacy in adjuvant therapy. **Scientific Reports**, v. 8, p. 1-12, 2018.

PINZ, M. et al. 4-phenylselenyl-7-chloroquinoline, a new quinoline derivative containing selenium, has potential antinociceptive and anti-inflammatory actions. **European Journal of Pharmacology**, v. 5, n. 780, p. 122-128, 2016.

PINZ, M. et al. Current advances of pharmacological properties of 7-chloro-4-(phenylselanyl) quinoline: Prevention of cognitive deficit and anxiety in Alzheimer's disease model. **Biomedicine & Pharmacotherapy**, p. 1006- 1014, 2018.

PRASAD, S. N.; MURALIDHARA. Evidence of acrylamide induced oxidative stress and neurotoxicity in *Drosophila melanogaster* – Its amelioration with spice active enrichment: Relevance to neuropathy. **NeuroToxicology**, v. 33, p. 1254-1264, 2012.

PRASAD, S. N.; MURALIDHARA. Neuroprotective effect of geraniol and curcumin in an acrylamide model of neurotoxicity in *Drosophila melanogaster*: relevance to neuropathy. **Journal of Insect Physiology,** v. 60, p. 7-16, 2014.

RALDÚA, D. et al. Targeting redox metabolism: the perfect storm induced by acrylamide poisoning in the brain. **Scientific Reports**, v. 10, n. 312, p. 1-12, 2020.

REIS, A. et al. 4-phenylselenyl-7-chloroquinoline, a novel multitarget compound with anxiolytic activity: Contribution of the glutamatergic system. **Journal of Psychiatric Research**, 84, p. 191-199, 2017.

REIS, A. Pharmacological modulation of Na+, K+-ATPase as a potential target for OXA-induced neurotoxicity: correlation between anxiety and cognitive decline and beneficial effects of 7-chloro-4-(phenylselanyl) quinoline. **Brain Research Bulletin**, v. 162, p. 282-290, 2020.

REYNOLDS, T. Acrylamide and cancer: Tunnel leak in Sweden prompted studies. **Journal of the National Cancer Institute**, v. 94, n. 12, p. 876-878, 2002.

SANSANO, M. et al. Dietary acrylamide: What happens during digestion. **Food Chemistry,** v. 237, p. 58-64, 2017.

SAVEGNAGO, et al. Synthesis and antioxidant properties of novel quinoline-chalcogenium compounds. **Tetrahedron Letters**, v. 54, p. 40-44, 2013.

SENTHILKUMAR, S. et al. Developmental and behavioural toxicity induced by acrulamide exposure and amelioration using phytochemicals in *Drosophila melanogaster*. **Journal of Hazardous Materials**, v. 394, 2020.

SENTKOWSKA, A.; PYRZYŃSKA, K. 2019. Investigation of antioxidant activity of selenium compounds and their mixtures with tea polyphenols. **Molecular Biology Reports**, v. 43, p. 3019-3024, 2019.

SHAKYA, B.; SIDDIQUE, Y. H. Exploring the neurotoxicity and changes in life cycle parameters of *Drosophila melanogaster* exposed to arecoline. **The Journal of Basic and Applied Zoology**, v. 79, n. 47, 2018.

SHARMA, C.; KANG, S. Garcinol pacifies acrylamide induced cognitive impariments, neuroinflammation and neuronal apoptosis by modulation GSK signaling and activation of pCREB by regulationg cathepsin B in the brain of zebrafish larvae. **Food and Chemical Toxicology**, p.1-14, v. 138, 2020.

SILVA, D. et al. 7-chloro-4-(phenylselanyl) quinoline co-treatment prevent oxidative stress in diabetic-like phenotype induced by hyperglycidic diet in *Drosophila melanogaster*. **Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology**, 2021.

STADLER, R. et al. Acrylamide from Maillard reaction products. **Nature**, v. 419, p. 449-450, 2002.

STEFANELLO, S. et al. Protective effects of novel organic selenium compounds against oxidative stress in the nematode *Caenorhabditis elegans*. **Toxicology Reports**, v. 2, p. 961-967, 2015.

TAREKE, E. et al. Acrylamide: a cooking carcinogen? **Chemical Research in Toxicology**, v. 13, n. 6, p. 517-522, 2000.

TAREKE, E. et al. Analysis of acrilamide, a carcinogen formed in heated foodstuffs. **Journal of Agricultural and Food Chemistry**, v. 50, p. 4998-5006, 2002.

TÖRNQVIST, M., EHRENBERG, L., HAGMAR, L. Leakage of acrylamide from a tunnel construction work: exposure monitoring and health effects to humans and animals. In: ALARA Network Workshop, Antwerp, Belgium, p.1-8, 2000.

UGUR, B.; CHEN, K.; BELLEN, H. *Drosophila* tools and assays for the study of human diseases. **Disease Models & Mechanisms**, v. 3, p. 235-244, 2016.

VOGT, A. et al. Organoselenium group is critical for antioxidant activity of 7-chloro-4-phenylselenyl-quinoline. **Chemico-Biological Interactions**, v. 282, p. 7-12, 2018.

WANG, J. et al. Down-regulation of telomerase reverse transcriptase-related anti-apoptotic function in a rat model of acrylamide induced neurobehavioral deficits. **Biotechnic & Histochemistry,** v. 93, p. 512-518, 2018.

YU, D. et al. Gestational exposure to acrylamide inhibits mouse placental development *in vivo*. **Hazardous Materials**, v. 367, p. 160-170, 2019.

ZAMANI, E. et al. A review of acrylamide toxicity and its mechanism. **Pharmaceutical and Biomedical Research,** v. 3, n. 1, p. 1-7, 2017.

ZAMANI, E. et al. In vitro study towards role of acrylamide-induced genotoxicity in human lymphocyte and the protective effect of l-carnitine. **Brazilian Archives of Biology and Technology**, v. 61, p.1-10, 2018.

ZHANG, L. et al. Exposure to acrylamide decreases noradrenergic axons in rat brain. **NeuroToxicology**, v. 78, p. 127-133, 2020.

ZHAO, M.; WANG, F.; HU, X.; CHEN, F.; CHAN, H. Acrylamide-induced neurotoxicity in primary astrocytes and microglia: Roles of the Nrf2-ARE and NF-κB pathways. **Food and Chemical Toxicology**, v. 106, p. 25-35, 2017.