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MAGNA SOTELO BARRIENTOS

EFEITO DO EXTRATO DE QUINOA SOBRE UM MODELO EXPERIMENTAL DE DOENÇA DE PARKINSON EM *Drosophila melanogaster*

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

Orientador: Prof. Dr. Gustavo Petri Guerra

Coorientador: Profª. Drª. Marina Prigol

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Muito obrigada!

RESUMO

A doença de Parkinson (DP) é uma doença neurodegenerativa caracterizada pela degeneração dos neurônios dopaminérgicos da substância negra no sistema nervoso central, além disso, alterações no sistema colinérgico são observados nesta patogênese, sendo que os neurotransmissores dopamina e acetilcolina exercem funções fisiológicas importantes. O estresse oxidativo parece ser um fator importante que contribui para o desenvolvimento DP. A rotenona, um composto tóxico inibidor do complexo I mitocondrial na cadeia de transporte de elétrons induz ao aumento de estresse oxidativo e consequentemente ocasiona danos celulares, levando à sintomas características apresentadas da DP. O medicamento utilizado no tratamento da DP não apresenta efeito de prevenção e ainda pose causar efeitos colaterais. Desta forma, torna-se importante a busca de tratamentos naturais eficientes e sem efeitos colaterais. A quinoa, uma importante fonte de compostos bioativos, como os compostos fenólicos mostra suas propriedades antioxidantes com ação protetora contra os danos celulares, que protege contra os fatores que podem levar ao desenvolvimento de doenças neurodegenerativas. Assim, o objetivo do trabalho foi avaliar o efeito do extrato de quinoa sobre modelo experimental tipo doença de Parkinson induzido por rotenona em *Drosophila melanogaster*. Moscas, machos e fêmeas com 1 a 4 dias de idade foram separas em 4 grupos e expostas durante 7 dias à dieta: controle; extrato de quinoa (5,0 mg/mL); rotenona (500 μM) e rotenona (500 μM) + extrato de quinoa (5,0 mg/mL). A avalição de consumo alimentar foi realizada em um único dia e a taxa sobrevivência foi monitorada a cada 24 horas durante 7 dias. Após foram realizadas avaliações comportamentais de geotaxia negativa, campo aberto e memória. Em seguida, foram realizadas avaliações bioquímicas dos indicadores de estresse oxidativo, níveis da dopamina, atividade das enzimas tirosina hidroxilase e acetilcolinesterase na cabeça das moscas. Constatou-se, nos testes comportamentais, as moscas expostas à rotenona mostraram diminuição na sobrevivência, deficiência nas atividades locomotoras e da memória, bem como, observou-se diminuição dos níveis da dopamina e da atividade das enzimas tirosina hidroxilase e acetilcolinesterase. Nos indicadores de estresse oxidativo, diminuíram a atividade do superóxido dismutase (SOD) e catalase (CAT) e, umentaram a produção de espécies reativas e o TBARS. Entretanto, a co-exposição ao extrato de quinoa protegeu a toxicidade ocasionada pela rotenona nas avaliações comportamentais e bioquímicas. Esta proteção pode estar relacionada aos compostos fenólicos presentes no extrato de quinoa, que apresentam mecanismo de ação antioxidante e capacidade de modular algumas enzimas. Estes resultados evidenciam ser uma possível coadjuvante no tratamento de DP.

Palavras-chave: Neurotoxicidade, antioxidantes, estresse oxidativo, compostos fenólicos, extrato natural.

ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease characterized by the degeneration of dopaminergic neurons of the substantia nigra in the central nervous system, in addition, changes in the cholinergic system are observed in this pathogenesis, and the neurotransmitters dopamine and acetylcholine exert important physiological functions. Oxidative stress appears to be an important factor contributing to PD development. Rotenone, a toxic compound I inhibitor of mitochondrial complex I in the electron transport chain, induces an increase in oxidative stress and consequently causes cellular damage, leading to the characteristic symptoms of PD. The medication used in the treatment of PD does not have a preventive effect and can still cause side effects. In this way, it becomes important to search for efficient natural treatments without side effects. Quinoa, an important source of bioactive compounds such as phenolic compounds, shows its antioxidant properties with protective action against cell damage, which protects against factors that can lead to the development of neurodegenerative diseases. Thus, the objective of this work was to evaluate the effect of quinoa extract on rotenone-induced Parkinson's disease experimental model in Drosophila melanogaster. Flies, males and females with 1 to 4 days old were separated into 4 groups and exposed for 7 days to the diet: control; quinoa extract (5.0 mg/mL); rotenone (500 µM) and rotenone (500 µM) + quinoa extract (5.0 mg/mL). The assessment of food consumption was performed on a single day and the survival rate was monitored every 24 hours for 7 days. After that, behavioral evaluations of negative geotaxis, open field and memory were performed. Then, biochemical evaluations of oxidative stress indicators, dopamine levels, activity of tyrosine hydroxylase and acetylcholinesterase enzymes were performed in the flies' heads. In behavioral tests, flies exposed to rotenone showed decreased survival, impaired locomotor activities and memory, as well as decreased levels of dopamine and activity of tyrosine hydroxylase and acetylcholinesterase enzymes. In the oxidative stress indicators, they decreased the activity of superoxide dismutase (SOD) and catalase (CAT) and increased the production of reactive species and TBARS. However, co-exposure to quinoa extract protected the toxicity caused by rotenone in behavioral and biochemical evaluations. This protection may be related to the phenolic compounds present in the quinoa extract, which have an antioxidant action mechanism and the ability to modulate some enzymes. These results show that it is a possible adjunct in the treatment of PD.

Keywords: Neurotoxicity, antioxidants, oxidative stress, phenolic compounds, natural extract.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

ACh - Acetilcolina

AChE - Acetilcolinesterase

CAT - Catalase

DA - Dopamina

DP - Doença de Parkinson

ERNs - Espécies reativas de nitrogênio

EROs - Espécies reativas de oxigênio

GABA -Ácido gama aminobutírico

H_2O_2 - Peróxido de hidrogênio

$HO^{\bullet}$ - Radical hidroxila

MAO - Monoamina oxidase

NO - Óxido nítrico

$O2^{-\bullet}$ - Radical ânion superóxido

ONOO - Peroxinitrito

SNC - Sistema nervoso central

SOD - Superóxido dismutase

TH – Tirosina hidroxilase

SUMÁRIO

1 INTRODUÇÃO

A doença de Parkinson (DP), descrita pela primeira vez por James Parkinson, em 1817, (PARKINSON, 2002). ADP é uma doença neurodegenerativa multifatorial que provoca um distúrbio no sistema nervoso central (HAYES, 2019). A estimativa em 2019 indica que a doença afeta cerca de 4 milhões de pessoas no mundo com idade acima de 65 anos, e mostra significativamente aumentada em idosos com mais de 80 anos (OU *et al.*, 2021), sendo o maior número em homens em comparação as mulheres (HAYES, 2019). A DP é caracterizada por sintomas motores como, tremores, rigidez muscular, transtornos do equilíbrio postural e bradicinesia (FRISARDI; SANTAMATO; CHEERAN, 2016; (HAYES, 2019), além disso, os pacientes com DP podem apresentar sintomas não motores, como ansiedade, psicose, comprometimento cognitivo, sendo que a demência apresenta maior relevância em pacientes com DP por mais de 20 anos (ROBBINS; COOLS, 2014; KALIA; LANG, 2015; LOTANKAR; PRABHAVALKAR; BHATT, 2017).

A alteração patológica comumente observada na doença é a degeneração progressiva dos neurônios dopaminérgicos na parte compacta da substância negra (LOTANKAR; PRABHAVALKAR; BHATT, 2017). A dopamina (DA) é um neurotransmissor que está envolvido no controle de várias funções, como cognição, motivação, movimento e recompensa e prazer (IVERSEN; IVERSEN, 2007; ROHEGER; KALBE; LIEPELT-SCARFONE, 2018).

A diminuição dos níveis da DA pode levar a um desequilíbrio da neurotransmissão dopaminérgica-colinérgica estriatal (YARNALL; ROCHESTER; BURN, 2011). A acetilcolinesterase (AChE) é a enzima responsável por hidrolisar o neurotransmissor acetilcolina (ACh) nas sinapses colinérgicas, onde atua transmitindo a mensagem de um neurônio a outro responsável por funções motoras e cognitivas (ARAÚJO, *et al.*, 2016).

Entretanto, as causas para DP, sejam relacionadas a fatores genéticos ou ambientais, ainda não estão completamente elucidadas (Hayes, 2019). Evidencias demonstram que o acúmulo de Corpos de Lewy, inclusões intraneuronais compostas principalmente por agregados proteicos de α-sinucleína, provocam a degeneração de neurônios dopaminérgicos, com consequente diminuição nos níveis de DA (JADIYA *et al.*, 2011; GOEDERT *et al.*, 2013). Evidências também demonstram que a disfunção do complexo I mitocondrial, com consequente aumento dos radicais livres, está

relacionado com a degeneração de neurônios dopaminérgicos (BENDER *et al.*, 2006; GREENAMYRE *et al.*, 2010; KRAYTSBERG *et al.*, 2006; SUBRAHMANIAN; LAVOIE, 2021). Neste sentido, fatores ambientais, como o uso de inseticidas e pesticidas, incluindo rotenona e paraquat podem estar associados com um aumento no desenvolvimento de DP (NISTICÒ *et al.*, 2011; SANDERS; TIMOTHY GREENAMYRE, 2013; KHATRI; JUVEKAR, 2016). A rotenona é um composto lipofílico e tóxico que atravessa facilmente a barreira sangue-cérebro, mostra ser um modelo importante na patogênese da DP (CICCHETTI; DROUIN-OUELLET; GROSS, 2009; BOVÉ; PERIER, 2012).

Os tratamentos para DP apresentam um efeito limitado, com uma melhora temporária dos sintomas, diminuindo seu efeito ao longo do tempo, além disso provoca efeitos colaterais, como, náusea, vômitos, hipotensão, taquicardia, midríase, insônia, depressão (OLANOW, 2008; OERTEL; SCHULZ, 2016)

Assim, o desenvolvimento de novas opções terapêuticas, sendo mais eficazes, seguras e que diminuam os efeitos colaterais, torna-se importante. Uma opção que surge são os compostos bioativos, constituintes extra nutricionais presentes nos alimentos de origem vegetal, com promissores efeitos benéficos para a saúde.

Os compostos bioativos, como os compostos fenólicos, são metabólitos secundários das plantas, quimicamente constituídos por anel aromático com um ou mais substituintes hidroxílicos (LAJOLO; MERCADANTE, 2018; VUOLO; LIMA; MARÓSTICA JUNIOR, 2019), estão presentes em maior proporção na parte externa do vegetal, mais de 10.000 tipos já foram identificados, sendo os mais abundantes os ácidos fenólicos e os flavonóides (LAJOLO; MERCADANTE, 2018). O interesse por esses compostos é devido ao potencial antioxidante, com efeito protetor contra os danos celulares e o desenvolvimento de doenças, incluindo doenças neurológicas (CARUANA; VASSALLO, 2015; UDDIN *et al.*, 2020). Em pesquisas, o estresse oxidativo induzido por compostos tóxicos mostram ser atenuados com tratamento através de extratos vegetais rica em compostos fenólicos, como o morango (GIAMPIERI *et al.*, 2016), Physalis (AREIZA-MAZO *et al.*, 2018) abacate (ORTEGA-ARELLANO; JIMENEZ-DEL-RIO; VELEZ-PARDO, 2019) e curcumina (BURATTA *et al.*, 2020).

A quinoa (*Chenopodium quinoa* Willd) é um pseudocereal da família Amaranthaceae, originária dos Andes de países da América do Sul (JACOBSEN; MUJICA; JENSEN, 2003), possui uma importante fonte compostos fenólicos, como, o

ácido cafeico, ácidos ferúlico, ácido *p*-cumárico, ácido vanílico, miricetina, quercetina, kaempferol, entre outros (REPO-CARRASCO-VALENCIA *et al.*, 2010; TANG *et al.*, 2015), em avaliações *in vitro* mostram elevada capacidade antioxidante (CARCIOCHI; MANRIQUE; DIMITROV, 2015; ABDERRAHIM *et al.*, 2015; TANG *et al.*, 2016), bem como, em estudos *in vivo* (SOUZA *et al.*, 2020). Entretanto, os diversos tratamentos realizados para seu consumo, como aplicação de torrefação e extrusão (BRADY *et al.*, 2007), lavagem (NICKEL *et al.*, 2016) e cocção (DINI; TENORE; DINI, 2010), podem levar à diminuição no conteúdo de compostos fenólicos, resultando na perda de propriedades antioxidantes. Sendo assim, a obtenção do extrato *in natura* da quinoa pode ser uma alternativa para o melhor aproveitamento dos compostos fenólicos e consequentemente uma maior eficácia sobre os efeitos farmacológicos e possível proteção contra doenças (SOUZA *et al.*, 2020).

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o efeito do extrato de quinoa (*Chenopodium quinoa* Willd) sobre a neurotoxicidade induzida por rotenona em um modelo experimental tipo doença de Parkinson em *Drosophila melanogaster*.

2.2 Objetivos Específicos

- Avaliar o efeito do extrato de quinoa sobre a taxa de sobrevivência e atividades locomotoras em *Drosophila melanogaster*.

- Avaliar a taxa de sobrevivência, atividades locomotoras e memória em *Drosophila melanogaster* expostas a rotenona e extrato de quinoa.

- Avaliar os níveis da DA e atividade das enzimas TH e AChE em *Drosophila melanogaster* expostas a rotenona extrato de quinoa.

- Avaliar os indicadores do estresse oxidativo em *Drosophila melanogaster* expostas a rotenona e extrato de quinoa.

3 REVISÃO BIBLIOGRÁFICA

3.1 Quinoa (Chenopodium quinoa Willd)

A quinoa (*Chenopodium quinoa* Willd) (Figura 1), é um pseudocereal da família amarantaceae, apresenta uma ampla variabilidade genética e adaptabilidade a diversos ambientes de cultivo, originária e cultivada nos andes dos países da América do Sul (JACOBSEN; MUJICA; JENSEN, 2003; REPO-CARRASCO; ESPINOZA; JACOBSEN, 2003). Após a década de 90 seu cultivo expandiu-se em países tropicais da América do Sul e outros continentes, como na Europa (JACOBSEN, S.-E., 2017), Ásia (HU *et al.*, 2017) e América do Norte (TESTEN *et al.*, 2014).

Figura 1- Planta de quinoa (esquerda); sementes de quinoa (direita)

(FAO; INIA, 2013)

A quinoa é um produto de alto valor nutricional em comparação a outros cereais, apresenta elevado teor de fibras, contêm carboidratos, aminoácidos essenciais como a lisina, proteínas, vitaminas, minerais e livre de glúten (STIKIC *et al.*, 2012; NOWAK; DU; CHARRONDIÈRE, 2016). Devido as características favoráveis para a saúde humana, a Organização das Nações Unidas para Alimentação e Agricultura, declarou no ano 2013 o Ano Internacional da Quinoa (FAO, 2013). Além das propriedades nutricionais, a quinoa possuiu um amplo número de compostos bioativos que são metabólitos secundários, como, ácidos fenólicos,

flavonóides, terpenóides, esteróides e compostos contendo nitrogênio, com funções fisiológicas e biológicas (LIN *et al.*, 2019).

Os metabólitos secundários são produzidos pelas plantas como mecanismo de defesa aos fatores que podem interferir no seu crescimento, é bem descrito que a quinoa nas diversas variedades mostra ser resistente ao ataque pelo estresse abiótico (HINOJOSA *et al.*, 2018). Em plantas, a seca pode diminuir a taxa fotossintética e a alta salinidade do solo é um dos principais fatores que podem levar à redução na fotossíntese, respiração, síntese de proteínas, fechamento estomático e alta produção de espécies reativas de oxigênio (GUPTA; HUANG, 2014). A quinoa, desenvolve diversos mecanismos para combater o estresse abiótico, na deficiência hídrica possui a capacidade de aumentar a produção de ácido abscísico (JACOBSEN; LIU; JENSEN, 2009; YANG *et al.*, 2016) e ao estresse provocado pela salinidade, mostra controle eficiente do sequestro de Na^+ nas células das plantas, maior tolerância a espécies reativas de oxigênio, melhor retenção de K^+, manutenção de baixos níveis citosólicos de Na^+ e uma alta taxa de bombeamento de H^+ na célula mesofílica (ADOLF; JACOBSEN; SHABALA, 2013; BONALES-ALATORRE *et al.*, 2013).

Outro fator do estresse abiótico é a temperatura alta, induz ao acúmulo de espécies reativas ao oxigênio, que causa séria toxicidade às plantas (WAHID *et al.*, 2007). A quinoa dependendo do genótipo mostra tolerância a uma faixa de temperaturas entre - 8 °C a 35 °C e condições de umidade relativa entre 40% a 88% (JACOBSEN *et al.*, 2005), porém, a temperatura pode afetar na produção de sementes, principalmente a alta temperatura reduz a produção de semente de quinoa (HINOJOSA *et al.*, 2018).

É destacado, que o acúmulo de compostos bioativos, como os fenólicos totais, está associado com a tolerância ao sal na quinoa, em um estudo com três variedades de quinoa com concentrações diferentes de sal (NaCl 100 e 300 mM), os resultados mostraram aumento de fenólicos totais nas três variedade e consequentemente mostra aumento da atividade antioxidante (ALOISI *et al.*, 2016). A quinoa apresenta alto teor de compostos fenólicos, evidentemente mais alto em flavonoides em comparação com outros pseudocereal, como a kaniwa (REPO-CARRASCO-VALENCIA *et al.*, 2010).

Em estudos *in vitro* determinados pelos métodos da eliminação de radicais DPPH, poder antioxidante redutor férrico (FRAC) e capacidade de absorção de radicais de oxigênio (ORAC), é demostrado que as sementes de quinoa das cores

vermelha e preta apresentam maior teor de compostos fenólicos e consequentemente maior capacidade antioxidante em comparação às sementes da cor branca (TANG; ZHANG; *et al.*, 2016; PEREIRA *et al.*, 2020). No trabalho de Souza et al. (2020), em uma avaliação *in vivo* , a administração do extrato de quinoa vermelha preveniu déficit de memória, alterações da atividade da enzima AChE e a toxicidade induzida por escopalamina no hipocampo e parcialmente no córtex cerebral em camundongos.

3.2 Compostos fenólicos

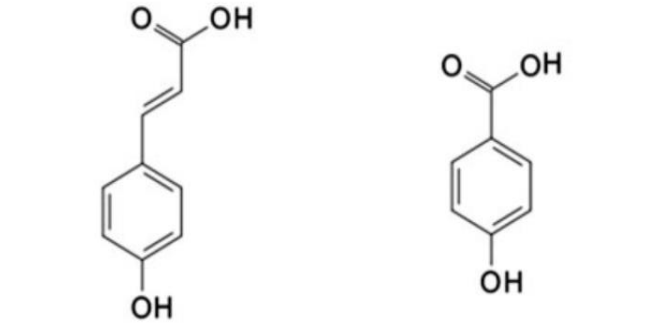
Os compostos fenólicos são metabólitos secundários, com propriedades antioxidantes sintetizadas pelas plantas como forma de proteção contra os danos bióticos e abióticos, bem como são responsáveis pelas características sensoriais, além disso, apresenta benefícios para a saúde humana (SINGLA *et al.*, 2019). Os compostos fenólicos estão presentes em cereais integrais (CĂLINOIU; VODNAR, 2018), frutas, hortaliças, legumes, folhas e em produtos de origem vegetal, como, café, chá e vinho (LAJOLO; MERCADANTE, 2018). O mecanismo da capacidade antioxidante é influenciada da estrutura do flavonoide (FL-OH), estando correlacionadas, com os grupos hidroxilas presentes no anel aromático e com a presença da ligação dupla entre moléculas de carbono (LAJOLO; MERCADANTE, 2018), bem como, possuem capacidade de quelar metais pro-oxidantes, capacidade sequestrar espécies reativas de oxigênio e nitrogênio, capacidade de modular certas enzimas do sistema antioxidante endógeno e de detoxificação (LAJOLO; MERCADANTE, 2018). Os compostos fenólicos são classificadas de acordo com a estrutura química, em: ácidos fenólicos, flavonóides, estilbenos, cumarinas e taninos, os compostos fenólicos mais abundantes em alimentos são ácidos fenólicos e flavonoides (LAJOLO; MERCADANTE, 2018).

3.2.1 Ácidos fenólicos

Dentre os ácidos fenólicos estão os derivados do ácido cinâmico e ácido benzóico que apresentam uma estrutura básica de nove átomos de carbono C6 – C3 e sete átomos de carbono C6 – C1 respectivamente, e, estão predominantemente na forma hidroxilada (Figura 2) (VUOLO; LIMA; MARÓSTICA JUNIOR, 2019). Os derivados do ácido hidroxibenzóico, são o ácido elágico e gálico estão presentes em

cereais integrais (NAYAK; LIU; TANG, 2015), e em diversas frutas da família *Myrtaceae* e *Rosaceae* (morango, goiaba, jabuticaba, camu-camu, amora-preta e romã) (LAJOLO; MERCADANTE, 2018), já os derivados dos ácidos hidroxicinâmicos, incluem, os ácidos *p*-cumárico, cafeico, sináptico e ferúlico, são encontrados em chá-mate e uva (LAJOLO; MERCADANTE, 2018) e cereais integrais (NAYAK; LIU; TANG, 2015).

Figura 2 - Exemplo da estrutura química de ácidos fenólicos

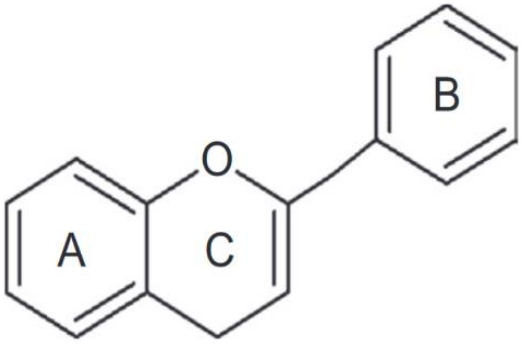


Fonte: ISSAOUI *et al* (2020, p. 3)

3.2.2 Flavonóides

Os flavonóides (Figura 3), estão compostos de uma estrutura básica de 15 carbonos, por dois anéis aromáticos (anel A e B), unidos por uma cadeia linear de três carbonos que pode formar um anel heterocíclico (anel C), onde o anel B pode se ligar nas posições 2, 3 ou 4 do anel C (VUOLO; LIMA; MARÓSTICA JUNIOR, 2019; LAJOLO; MERCADANTE, 2018). Os flavonóides se subdividem em subclasses, entre os compostos encontrados em maior quantidade em alimentos estão os flavonóis, flavonas, flavan-3-oís, antocianinas, flavanonas e isoflavonas (LAJOLO; MERCADANTE, 2018).

Figura 3 - Exemplo da estrutura química dos flavonóides



Fonte: VUOLO; LIMA; MARÓSTICA JUNIOR (2019, p. 38)

3.3 Estresse oxidativo

O estresse oxidativo foi definido por Helmut Sies, em 1985, como um desequilíbrio entre pró-oxidantes e antioxidantes (SIES, 2015). Posteriormente, foi redefinido como uma interrupção da sinalização e controle redox, sustentando que os principais sistemas celulares não estão em equilíbrio redox e respondem de forma diferente a tóxicos químicos e estímulos fisiológicos (JONES, 2006). A homeostase celular é mantida pelo equilíbrio redox, dada pela geração e eliminação de espécies reativas de oxigênio (ERO_S) e nitrogênio (ERN_S), dentro do ERO_S estão o radical ânion superóxido (O2−•), radical hidroxila ($HO^{\bullet}$) e peróxido de hidrogênio (H_2O_2) (SIES; BERNDT; JONES, 2017).

A formação de espécies reativas pode ser contribuída por fontes exógenas como a irradiação, poluentes e produtos químicos e, a fonte principal endógena de ROS é a mitocôndria (TRACHOOTHAM *et al.*, 2008). Em células aeróbicas, as mitocôndrias da cadeias de transporte de elétrons pelos complexos I, II e III, são a principal fonte de ERO_S, produzem $O_2^{-\bullet}$ e H_2O_2 (SIES; BERNDT; JONES, 2017).

Entretanto, o organismo desenvolve enzimas antioxidantes e estão presentes simultaneamente nas células e tecidos, são dependentes da concentração de O_2 e de sinais exógenos de naturezas físicas ou químicas, bem como, do tipo específico de célula, tecido ou órgão (SIES; BERNDT; JONES, 2017).

As enzimas responsáveis por manter o nível fisiológico ante a formação de espécies reativas, são: superóxido dismutase (SOD) que catalisa a dismutação do produto inicial superóxido $O_2^{-\bullet}$ em H_2O_2 (WANG *et al.*, 2018), seguida pela atividade

da catalase (CAT), que converte o H_2O_2 em $H_2O + O_2$, uma enzima à base de heme que normalmente está localizada no peroxissomo e possui alta taxa de renovação de substrato (GLORIEUX; CALDERON, 2017). Outras enzimas com funções desintoxicantes no sistema redox, como a enzima glutationa S-transferase, possui a capacidade de desintoxicar eletrofílicos biologicamente reativos gerados pelo citocromo P-450 e a enzima quinona redutase que atua na desintoxicação de quinonas (SIES; BERNDT; JONES, 2017)**.** O aumento excessivo na produção de ERO_S tem sido implicado em várias consequências do envelhecimento, e na patogênese de várias doenças, como, câncer, diabetes mellitus, cardiovasculares, doenças neurodegenerativas, artrite reumatóide, entre outras (DRÖGE, 2002; JONES, 2006).

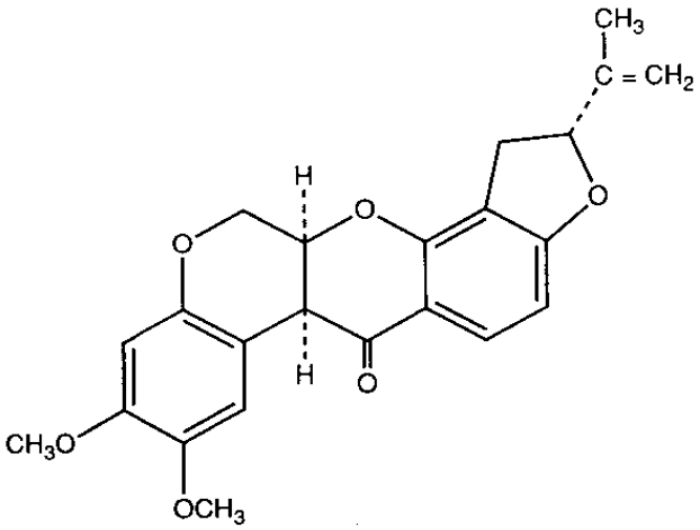
3.4 Rotenona

Rotenona ([2R-(2α,6aα,12aα)]-1,2,12,12a-tetra-hidro-8,9-dimetoxi-2-(1-metiletenil)-[1]benzopirano[3,4-b]furo [2,3-h][1]benzopirano-6(6aH)-ona) (Figura 4), é um composto natural derivado das raízes e caules das espécies de plantas *Derris, Tephrosia, Lonchocarpus* e *Mundulea* (RADAD *et al.*, 2019). Apresenta característica altamente lipofílica e tóxica devido a facilidade de atravessa a barreira sangue-cérebro (CICCHETTI; DROUIN-OUELLET; GROSS, 2009).

Há centenas de anos, raízes das plantas que continham o composto rotenona tem sido utilizado na pesca pelos nativos e viajantes nas Índias orientais, América do Sul e África tropical (LA FORGE; HALLER; SMITH, 1933). No ano 1902,o químico japonês Nagai Nagayoshi, isolou um composto puro cristalino a partir planta *Derris elliptica* e identificou como rotenona (LA FORGE; HALLER; SMITH, 1933). A rotenona é um pesticida seletivo, apresenta ação inseticida e piscicida, foi amplamente utilizado em árvores frutíferas e no controle da população de peixes (WORLD HEALTH ORGANIZATION, 1992; LING, 2003). Após 1990, a rotenona como pesticida agrícola deixou de ser usado e, posteriormente passou a ser de uso restrito pela Agência de proteção Ambiental dos Estados Unidos (USEPA) devido ao efeito tóxico relacionado a doenças neurológicas (LAWANA; CANNON, 2020). Nos EUA ainda é utilizado, especificamente para o controle de peixes nos lagos e rios, bem como, a venda somente é autorizada para aplicadores certificados (LAWANA; CANNON, 2020).

A Organização Mundial da Saúde, indica que a rotenona é altamente tóxica para a vida aquática, entretanto, no meio ambiente é facilmente oxidado em outros compostos menos tóxicos, bem como apresenta baixa toxicidade em aves, e para os seres humanos a rotenona pura é considerara moderadamente tóxica (WORLD HEALTH ORGANIZATION, 1992). A absorção da rotenona pode ocorrer por ingestão e inalação, através da pele é considerada baixa, é metabolizado do fígado e eliminada pelas fezes (WORLD HEALTH ORGANIZATION, 1992).

Figura 4 - Estrutura química da rotenona



Fonte: WORLD HEALTH ORGANIZATION (1992, p. 7)

A toxicidade da rotenona é devido a ação inibidora do complexo I mitocondrial, a administração via oral é menos tóxica para o camundongo e hamster do que para o rato, e mostra ser mais tóxica em ratos fêmeas do que para os machos (WORLD HEALTH ORGANIZATION, 1992). Pesquisas utilizando modelos animais mostram a toxicidade da rotenona uma característica observada na DP. Em ratos injetados com rotenona no feixe do prosencéfalo mediano esquerdo, a rotenona atua como um inibidor potente e seletivo do complexo I da cadeia de transporte de elétrons e diminuição significativa do nível de DA (HEIKKILA *et al.*, 1985). Em uma avaliação histológica de cortes corados em todo o cérebro de ratos tratados com rotenona resultou em lesões bilaterais seletivas dentro do corpo estriado e globo pálido (FERRANTE *et al.*, 1997). A inibição crônica do complexo I pela rotenona em ratos, causa degeneração dopaminérgica nigroestriatal e acumulação de inclusões citoplasmáticas fibrilares que contêm ubiquitina e α-sinucleína (BETARBET *et al.*,

2000), ainda, uma exposição crônica à rotenona em ratos pode levar a uma modificação oxidativa da proteína DJ-1, acúmulo de a α-sinucleína e comprometimento proteassomal (BETARBET *et al.*, 2006). Em estudo utilizando *Drosophila melanogaster*, a exposição à rotenona ocasiona alterações na expressão de mRNA, na atividade das enzimas antioxidantes SOD e CAT e no gene da TH (SUDATI *et al.*, 2013), como também, a exposição de *Drosophila melanogaster* à rotenona, mostra alterações na enzima desintoxicante Glutatiora S-transferase, na enzima AChE, e ocasiona diminuição dos níveis da DA (ARAUJO *et al.*, 2015; DE FREITAS COUTO *et al.*, 2019; FERNANDES *et al.*, 2021). A disfunção do complexo I mitocondrial está caracterizado no aumento de estresse oxidativo, considerado um papel importante na degeneração dos neurônios dopaminérgicos, principal sinal observada na DP (BENDER *et al.*, 2006; HENCHCLIFFE; BEAL, 2008).

3.5 Doença de Parkinson

A DP foi descrita como síndrome específica a mais de 200 anos por James Parkinson em 1817 em “An Essay on the Shaking Palsy”, e ainda continua persistindo (PARKINSON, 2002). A DP é uma doença crônica e progressiva, que causa afecção degenerativa do sistema nervoso central, com amplo espectro, principalmente de sintomas motor (FRISARDI; SANTAMATO; CHEERAN, 2016) e está associada a uma variedade de sintomas não motores, como, ansiedade, psicose e a capacidade cognitiva (THENGANATT; JANKOVIC, 2014; LOTANKAR; PRABHAVALKAR; BHATT, 2017).

A DP é a segunda doença neurodegenerativa mais prevalente na população mundial, afetando a mais de 4 milhões de indivíduos (DORSEY *et al.*, 2018). Além disso, atinge cerca de 1% dos idosos acima dos 65 anos de idade, e estima-se que 5% da população com mais de 85 anos de idade são afetadas (SHULMAN; DE JAGER; FEANY, 2011). Etiologicamente, a DP é complexa e envolve fatores genéticos, embora a maioria dos casos parece ser multifatorial como, fatores genética e toxinas ambientais (SCHAPIRA, 2010), e imunológicos (DE VIRGILIO *et al.*, 2016).

A DP caracteriza-se pela degeneração dos neurônios dopaminérgicos na região compacta da substância negra, ocasionando depleção de DA (DAUER; PRZEDBORSKI, 2003; GAO; WU, 2016), levando a um aumento de ACh, ocasionando desequilíbrio entre os neurotransmissores (YARNALL; ROCHESTER;

BURN, 2011). A região do estriado, além da DA, está regulada por uma variedade de neuro-moduladores, como a ACh, que apresenta função excitatória dos neurônios envolvidos no controle do movimento e cognitivo (LV *et al.*, 2017). Bem como, a fisiopatologia da DP ainda apresenta os corpos de Lewy, um acumulo intracelular de conformações oligoméricas de agregados de α-sinucleína (GOEDERT *et al.*, 2013), que são protofibrilas com potencial toxicológico, que levam ao rompimento da homeostase celular, contribuindo para a morte celular, e conjunto dessas alterações específicas, indicam para um diagnóstico definitivo da DP (STEFANIS, 2012; POEWE *et al.*, 2017).

Devido a perda de DA no corpo estriado, ocorre aumento da atividade nos circuitos globo pálido interno (GPi) do corpo estriado ventral, e da porção reticulada da substância negra (SNpr), levando a inibição do tálamo devido a disfunção na liberação do ácido gama aminobutírico (GABA), consequentemente diminuição da função do tálamo em ativar o córtex frontal, levando à diminuição da função motora presentes na DP, que ocorre quando 50% a 80% dos neurônios dopaminérgicos da substância negra destruídos (DEMAAGD; PHILIP, 2015; RODRIGUEZ-OROZ *et al.*, 2009; SHULMAN; DE JAGER; FEANY, 2011; SONG *et al.*, 2017).

Clinicamente a DP é caracterizada em estágios iniciais pelo aparecimento de sintomas não motores, como disfunção olfatória, depressão e distúrbios do sono, declínio cognitivos que podem preceder até 10 anos antes do aparecimento dos sintomas motores (CHAUDHURI *et al.*, 2011; RANA *et al.*, 2015). Porém, esses sintomas dificilmente são identificados e associados com a DP (LEE; KOH, 2015). Em estágios mais avançados, surgem os sintomas motores clássicos caracterizados como uma síndrome clínica descrita como “parkinsonismo” que incluem tremor de repouso, bradicinesia, rigidez muscular e instabilidade postural (FAHN, 2018).

Os sintomas podem ser classificados como bradicinesia, alteração mais importante na DP, que corresponde a uma acentuada e gradual lentidão dos movimentos corporais; rigidez muscular devido a resistência involuntária e independente da velocidade ao movimento das articulações; tremor de repouso, que ocorre em baixa frequência em um membro em repouso e ausente durante o movimento; instabilidade postural, presente em estágios avançados da doença e que compromete a capacidade de caminhar ou manter-se em pé (ARMSTRONG; OKUN, 2020). A DP apresenta complexidade devido a impossibilidade de um diagnóstico definitivo e preciso nos estágios iniciais e tratar os sintomas em estágios mais

avançados (KALIA; LANG, 2015). Para o diagnóstico clínico de DP durante a vida, baseia-se na neurodegeneração, assim como nos sintomas motores, é primordial verificar a presença da bradicinesia para diferenciar a DP de outras patologias, ainda, a resposta positiva ao uso de levodopa também é usada para se ter diagnóstico mais preciso de DP (HUGHES *et al.*, 1992; CABREIRA; MASSANO, 2019.

Desta forma, observa-se que a síntese e atividade normal de DA é imprescindível para o funcionamento do sistema nervoso, sendo, a degeneração de DA pode ocasionar aumento de ACh, e, as alterações destes neurotransmissores estão associadas a muitas doenças, dentre elas doenças neurodegenerativas. Em organismos modelos, como a *Drosophila melanogaster* é amplamente estudada as atividades envolvidas, como a funções locomotoras e cognitivas, níveis da dopamina, atividade da AChE, relacionada à doenças neurodegenerativa, incluindo a DP (ARAUJO *et al*., 2015; DE FREITAS COUTO *et al*., 2019; FERNANDES *et al*., 2021).

3.5.1 Neurotransmissor dopamina

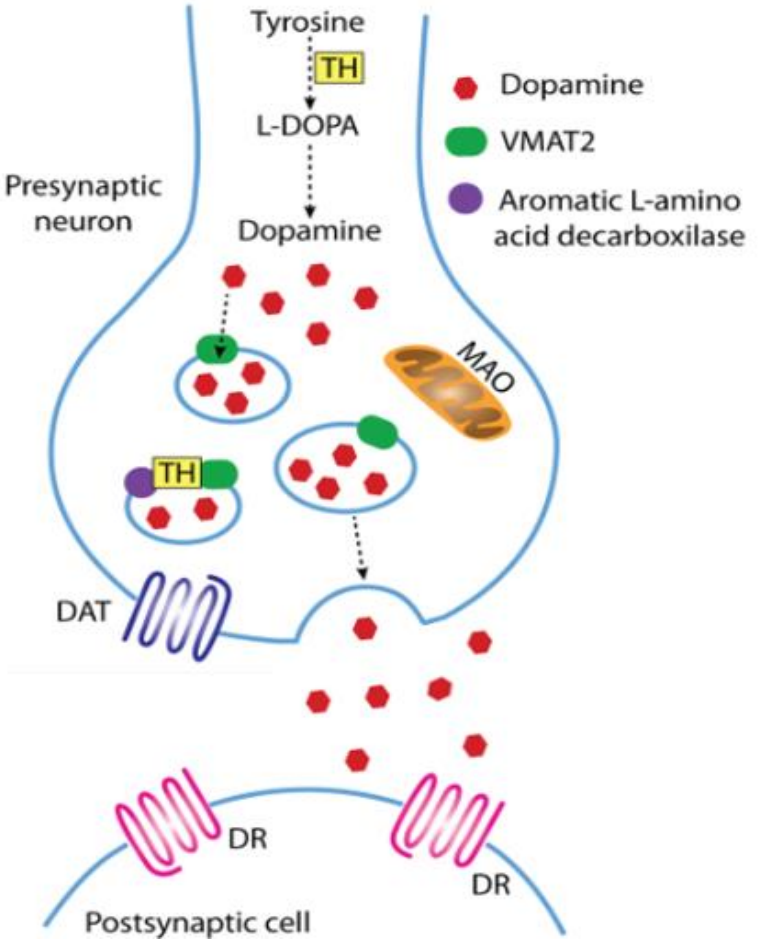
O neurotransmissor DA é sintetizado em regiões no sistema nervoso central e periférico. No sistema nevoso central, a substancia negra libera o neurotransmissor DA no estriado, que correspondem o núcleo caudado putamen, estriado ventral e núcleo acumbens (SCHULTZ, 1998). Já no sistema nervoso periférico é produzido pelos órgãos mesentéricos (EISENHOFER *et al.*, 1997).

A síntese da DA é iniciada pela enzima tirosina hidroxilase, utilizando cofatores tetrahidrobiopterina (BH4), oxigênio e ferro, que converte a tirosina em L-3,4-dihidroxifenilalanina (L-DOPA), posteriormente é convertida em dopamina pela descarboxilase de L-aminoácidos aromáticos (DOPA descarboxilase) e, o transportador vesicular de monoaminas (VMAT2), transfere e armazena nas vesículas sinápticas até sua liberação na fenda sináptica (KLEIN *et al.*, 2019; NAGATSU *et al.*, 2019) (Fig. 5). O sistema dopaminérgico desempenha funções importantes, como, o controle motor, função cognitivo, sono (MATSUMOTO *et al.*, 1999) (DOYA, 2000), motivação (SALAMONE; CORREA, 2012), recompensa e prazer (HORVITZ, 2000), comportamentos maternos e reprodutivos (KLEIN *et al.*, 2019).

Por sua vez, a DA pode sofrer oxidação enzimática e auto-oxidação. A oxidação enzimática ocorre no citosol pela ação da enzima monoamina oxidase

(MAO), que catalisa a desaminação oxidativa do grupo amino da dopamina em 3,4-dihidroxifenilacetaldeído levando à formação de amônia e peróxido de hidrogênio (SEGURA-AGUILAR *et al.*, 2014). A auto-oxidação, é a oxidaçã da dopamina em *o*-quinonas na ausência de catalisadores de íons metálicos sob condições aeróbicas, a *o*-quinona apresenta instabilidade em pH fisiológico, devido que o grupo amino da dopamina sofre ciclização para gerar leucoaminocromo, consequentemente sofre oxidação formando aminocromo (SEGURA-AGUILAR *et al.*, 2014).

Figura 5 - Desenho esquemático da síntese de dopamina



Fonte: KLEIN *et al* (2019, p.34)

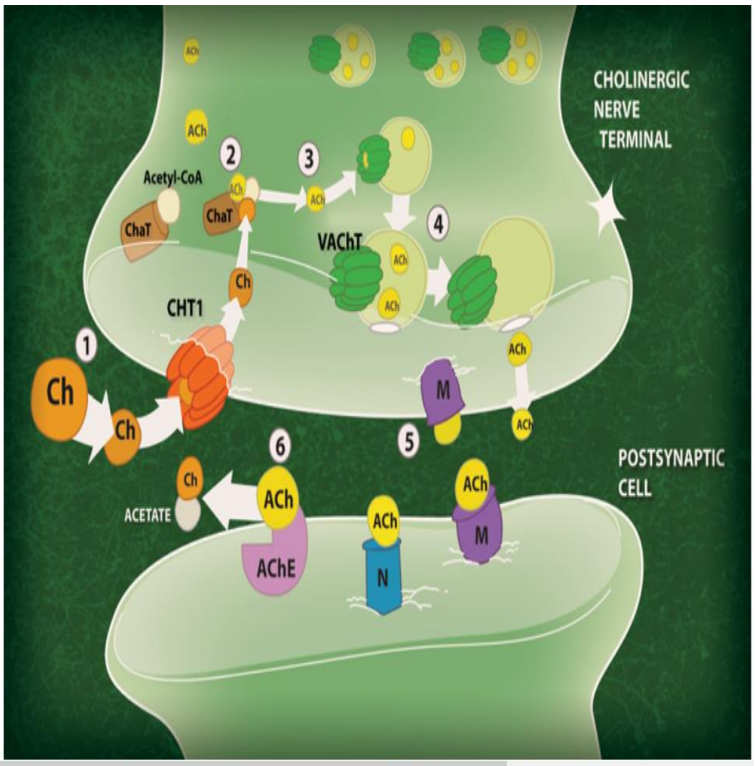
3.5.2 Enzima acetilcolinesterase

A enzima AChE é responsável pela hidrólise do neurotransmissor ACh (MILATOVIC; DETTBARN, 1996; ARAÚJO; SANTOS; GONSALVES, 2016). A ACh

se expande no sistema nervoso central e periférico, no cérebro, o sistema colinérgico se estende pela região córtex cerebral, que incluem os principais fontes, prosencéfalo basal, núcleo basal de Meynert e no corpo estriado (SELDEN, 1998), e está envolvido no comprometimento cognitivo e locomotor (VAN DER ZEE *et al.*, 2021).

A síntese da ACh ocorre no neurônio pré-sináptico a partir da reação colina e acetilcoenzima A (Acetil-CoA), após a formação é transferida pela colina acetiltransferase e armazenada em vesículas, mediante estímulo é liberada na fenda sináptica e transmite informação ao se ligar ao receptor pós-sináptico (PRADO *et al.*, 2013; ARAÚJO; SANTOS; GONSALVES, 2016), após a transmissão da mensagem, a ACh se desliga do receptor pós-sináptico, onde a enzima AChE catalisa dando origem em ácido acético e colina (ARAÚJO; SANTOS; GONSALVES, 2016) (Figura 6). A transmissão das mensagens entre os neurônios é realizada pelos receptores muscarínicos e nicotínicos (ZHOU; WILSON; DANI, 2002), sendo que os receptores muscarínicos de ACh exercem a função nos processos cognitivos, motores e comportamentais (LV *et al.*, 2017; CRANS *et al.*, 2020).

Figura 6 - Desenho esquemático de armazenamento e liberação de ACh



Fonte: PRADO *et al* (2013, p. 266)

4 MANUSCRITO CIENTÍFICO

Os resultados os quais fazem parte desta dissertação apresentam-se sob a forma de manuscrito científico, o qual encontra-se aqui estruturado sob o título: "Quinoa extract restored behavioral damage and neurotoxicity induced by Parkinson's disease-like model in *Drosophila melanogaster*"

Os itens Materiais e Métodos, Resultados e Discussão encontram-se inseridos no próprio manuscrito.

Quinoa extract restored behavioral damage and neurotoxicity induced by a Parkinson's disease-like model in *Drosophila melanogaster.*

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Abstract

Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons caused by several factors, including oxidative stress. Quinoa has an important source of phenolic compounds with antioxidant properties. In this work, the action of quinoa extract on locomotor damage and rotenone-induced neurotoxicity in *Drosophila melanogaster* was evaluated. Male and female fruit flies from 1 to 4 days old were exposed to a diet containing rotenone and/or quinoa extract for 7 days. Analyses of survival percentage, negative geotaxis, open field, memory, and food consumption were performed in vivo. After the behavioral tests, dopamine levels, tyrosine hydroxylase and AChE activities, and oxidative stress indicators were evaluated. Our results show that the exposure of flies to quinoa extract protected against mortality, locomotor impairment, and memory deficit induced by rotenone. Furthermore, quinoa extract restored dopamine levels, tyrosine hydroxylase and AChE activities, and oxidative stress indicators (ROS, TBARS, CAT, and SOD) after exposure to rotenone. The results suggest that the protective effect of quinoa extract in a model such as DP in Drosophila melanogaster may be related to its antioxidant potential and its ability to modulate enzymes.

Keywords: Neurodegenerative disease, neurotransmitters, oxidative stress, phenolic compounds, natural extract.

1 INTRODUCTION

Parkinson's disease (PD) is a multifactorial neurodegenerative disease that causes changes in the central nervous system, mainly affecting the performance of movements (LOTANKAR; PRABHAVALKAR; BHATT, 2017; COOLS *et al.*, 2019). This disease affects approximately 4 million people over 60 years old on a worldwide basis (HAYES, 2019). Characteristic motor symptoms of PD consist of tremors, muscle rigidity, postural balance disorders, and bradykinesia (FRISARDI; SANTAMATO; CHEERAN, 2016; HAYES, 2019). The inability of patients with PD to control movements is developed due to neurochemical alterations that affect the central nervous system, such as the accumulation of Lewy bodies (GOEDERT *et al.*, 2013), followed by progressive degeneration of dopaminergic neurons in the pars compacta of the substantia nigra (LOTANKAR; PRABHAVALKAR; BHATT, 2017), an area responsible for producing dopamine in the brain (COOLS *et al.*, 2019). However, other areas of the brain and molecular systems are affected, including, non-dopaminergic neurons. Evidence has shown mitochondrial dysfunction, increased oxidative stress (BENDER *et al.*, 2006; GREENAMYRE *et al.*, 2010), and changes in the cholinergic system associated with DP (CRANS *et al.*, 2020b). Altogether, these events are associated with disease progression, causing movement disorder as well as the appearance of non-motor symptoms, such as anxiety, psychosis, cognitive impairment, and memory deficit (ROBBINS; COOLS, 2014).

The wide variety of molecular systems affected in PD, with consequent diversity of symptoms, makes it difficult to have efficient treatments. Therefore, getting acquainted with discoveries and therapeutic options that truly modify the progression of PD symptoms is extremely important. In this sense, there has been a growing interest in alternative therapies which focus on the neuroprotective and antioxidant effects of bioactive compounds.

Quinoa (*Chenopodium quinoa* Willd) is a pseudocereal with high phenolic-compound content (TANG *et al.*, 2016; SOUZA *et al.*, 2020), especially when compared to other pseudocereals as kañiwa (*Chenopodium pallidicaule*) and kiwicha (*Amaranthus caudatus*) (REPO-CARRASCO-VALENCIA *et al.*, 2010). Phenolic compounds are secondary plant metabolites with antioxidant activity and the ability to scavenge free radicals (LAJOLO; MERCADANTE, 2018; CSEPREGI; HIDEG, 2018). Evidence supports that phenolic-compound consumption can be associated with a protective effect against cell damage (GIAMPIERI *et al.*, 2016; ORTEGA-ARELLANO;

JIMENEZ-DEL-RIO; VELEZ-PARDO, 2019) and a decrease in the risk of developing neurodegenerative disorders, such as PD (ARYAL *et al.*, 2020). Furthermore, oral administration of quinoa extract prevents memory deficit and fully restores alterations in the acetylcholinesterase (AChE) activity, as well as oxidative stress indicators induced by scopolamine in rats (SOUZA *et al.*, 2020). In this sense, quinoa arises as an alternative for the development of pharmacological treatments and new effective therapeutic options able to prevent neurodegenerative diseases. Therefore, the aim of this study was to investigate the neuroprotective effect of quinoa extract on locomotor and exploratory damage and neurotoxicity induced by rotenone in *Drosophila melanogaster*, as well as possible alterations in oxidative stress, AChE activity and dopamine levels.

2 MATERIAL AND METHODS

2.1 Chemicals

The red quinoa seed was purchased at the local market, and was used to obtain the ethanolic extract by the Soxhlet method. Obtaining and characterizing the quinoa extract were previously published by Souza et al. (2020). Rotenone was purchased from Sigma-Aldrich (St. Louis, MO, USA) and diluted in ethanol 98%. All the other reagents used were of analytical grade.

2.2 Drosophila melanogaster stock

The fruit flies (*Drosophila melanogaster* wild-type - Harwich strain) were obtained from the National species Stock Center (Bowling Green, Ohio, USA), and kept for about 4 days in glass bottles in the BOD incubator, under controlled light conditions (12-hour light / dark cycle, temperature (25 ± 1 ° C) and 60% humidity, and fed with 10 mL of standard food comprising corn flour (76.59%), wheat germ (8.51%), sugar (7.23%), powdered milk (7.23%), salt (0.43%), an 0.08% v/w methylparaben, and a pinch of dry yeast, until the treatment was finished.

2.3 Experimental protocol

2.3.1 Experiment 1: Effect of quinoa extract on locomotor and exploratory activity and survival percentage

A concentration-response curve for quinoa extract was performed to evaluate the effect on locomotor and exploratory activity and to define the concentration for subsequent experiments. Flies of both sexes from 1 to 4 days old were divided into four groups (50 flies in each group) and exposed either to a standard diet (control group) or a diet containing quinoa extract (1.5, 5.0, and 15.0 mg/mL) for 7 days. Afterward, the flies were submitted to negative geotaxis and open field tasks to evaluate locomotor and exploratory activity. Furthermore, the survival percentage was assessed during 7 days of exposure. The standard diet consisted of 1% w/v yeast beer, 2% w/v sucrose, 1% w/v milk powder, 1% agar w/v, and 0.08% w/v methylparaben. The treatment schedule is depicted in Figure 1A.

2.3.2 Experiment 2: Effect of quinoa extract on rotenone-induced behavioral damage, dopamine levels, tyrosine hidroxylase (TH) and acetylcholinesterase (AChE) activities, and oxidative stress indicators.

After experiment 1 determined that quinoa extract at a concentration of 5.0 mg/mL did not alter the locomotor activity of *Drosophila melanogaster*, we assessed whether this concentration of quinoa extract would protect against damage induced by rotenone. The flies were divided into four groups (50 flies each) and exposed either to a standard diet (control), a diet containing rotenone (500 µM), quinoa extract (5.0 mg/mL) or rotenone (500 µM) + quinoa extract (5.0 mg/mL) for 7 days. Afterward, the flies were subjected to negative geotaxis and open field tasks to evaluate locomotor and exploratory activity and aversive phototaxis to assess memory. In addition, survival percentage and food consumption were evaluated during 7 days of exposure. The concentration of rotenone (500 µM) was selected based on a previous study (SUDATI *et al.*, 2013). The total food medium in all groups contained a volume of 0.5% ethanol. After the behavioral tests, the flies' dopamine levels were determined, as well as TH and AChE activities, and oxidative stress indicators (ROS, TBARS, SOD, CAT, and GST). The treatment schedule is shown in Figure 2A.

2.4 In vivo assays

2.4.1 Survival

The survival rate was evaluated by counting the number of living flies once a day until the end of the experimental period (7 days). In the survival data, 250 flies per group were included and the total number of flies represents the sum of five independent experiments (50 flies per treatment). Data were expressed as the percentage of surviving flies.

2.4.2 Negative geotaxis test

The climbing capacity of the flies was evaluated through the negative geotaxis task as described by Jimenez del Rio; Guzman-Martinez; Velez-Pardo (2010), with some modifications. In summary, after 7 days of treatment, 25 flies from each group were individually placed in a vertical test tube, with a diameter of 1.5 cm, and gently tapped to the bottom of the tube. The time spent by each fly to reach the mark of 8 cm at the top of the tube was recorded. The test was repeated five times for each fly, with an interval of 1 min. Each fly had a maximum time of 120 s to scroll the apparatus until reaching 8 cm of height. During the test, the flies remained in their respective tubes and the time average was calculated individually for the statistical analysis. Five independent experiments were performed.

2.4.3 Open field test

Locomotor activity was evaluated through the open field test, performed according to Connolly (1966), with adaptations from Musachio et al. (2020). Five flies from each group were anesthetized on ice and placed individually in a Petri dish. The Petri dish was divided into quadrants (1 cm^2). Then, after 2 minutes of recovery from anesthesia and acclimatization, the number of crossings was determined during a 60-second-period. The test was performed in duplicate on a fly from each experimental group. Five independent experiments were performed.

2.4.4 Memory Assessment

The learning and memory of flies were assessed through the method described by Ali et al. (2011), using T-mazes with a dark side and a lit side, which contained 1µM of quinine for the training. Eighteen flies of both sexes from 1 to 4 days old were used per group. First, the phototaxic flies were selected by placing them on the dark side of

the maze and choosing the flies that moved towards the lit side. Then, those flies were submitted to training, which was performed in a maze containing quinine on the lit side, as previously described, carrying out 9 trials with each fly for 1 minute. Immediately after training, each fly was transferred to another maze that also had a dark side and a lit one but did not contain quinine, and 5 tests of 30 seconds were performed to verify whether the flies would still move towards the light or if they would remember the aversive substance they had experienced during the training. This memory behavior was recorded as PC0 (0 hours post-conditioning), and the flies were placed on standard food for 6 hours. After that period, the test was repeated, with 5 attempts of 30 seconds for each fly, and recorded as PC6 (6 hours post-conditioning). This test is considered an indicator of short-term memory.

2.4.5 Determination of food consumption

Food consumption was assessed according to Sun et al. (2013) with some modifications. From each group, 25 flies were separated and 5 of them were placed in a tube with food that had a final concentration of 0.5% FCF Brilliant Blue dye (FD & C Blue Dye no. 1) for 30 min. After the feeding period, each group of flies was euthanized on ice and had its head and body separated and washed. Then, 5 bodies of flies of each group were homogenized in 200 µL of distilled water and centrifuged at 14.000-x g for 2 min. The supernatant was measured with a 625 nm microplate reader. The optical density of the homogenates of the flies that consumed the diet without dye was used to generate a standard curve. Five independent experiments were performed. The consumption test is performed to eliminate the bias of the flies not consuming the diet that contained quinoa extract or rotenone.

2.5 Ex vivo assays

2.5.1 Sample Preparation

Immediately after the behavioral evaluations, the flies' heads were homogenized in a HEPES buffer solution (20 mM, pH 7.0), 10:1 (flies/volume µL) for 2 min. The resulting homogenate was then centrifuged at 10,600-x g for 10 min at 4 ^{o}C, and the supernatant fraction (S1) was used for the determination of dopamine levels, TH and AChE activities, and enzymatic and non-enzymatic indicators of oxidative stress. Each sample was analyzed in duplicate. The protein content was measured

colorimetrically using the Bradford method (BRADFORD, 1976) and bovine serum albumin (1 mg/mL) was used as standard.

2.5.2 Determination of dopamine levels by HPLC-DAD

To determine DA levels using high-performance liquid chromatography (HPLC), 30 heads of flies per treatment group were homogenized in 0.9% NaCl (288 µL) and 0.5 M HCl (12 µL) for 1 min. The homogenates were centrifuged for 10 min at 7.000-x g at 4°C. In the 0.22 µm PTFE filters, the supernatant was filtered and stored at 80°C until used. The samples supernatant (20 µL) was injected into the HPLC system using an automatic sampling device. The HPLC system consisted of a vacuum degasser and a quaternary pump connected to a reverse-phase column (Synergi 4 µm Fusion-RP 80 Å 4.6 x 250 mm; Phenomenex) coupled to a diode array detector (DAD). The mobile phase consisted of methanol, and water (12:88 v/v) adjusted to pH 3 with phosphoric acid, and the flow rate was maintained at 0.8 mL 1 min^{-1} (BIANCHINI *et al.*, 2019). Detection was performed at 198 nm and the results of dopamine levels were expressed in µg DA/mg of tissue. Five independent experiments were performed.

2.5.3 Determination of tyrosine hydroxylase (TH) activity

TH activity was measured according to the method described previously by Vermeer et al. (2013), with some modifications, using 30 heads of flies per group. The reaction medium was prepared with 100 µL sample supernatant, 50 µM tyrosine, and 100 µM sodium periodate. The reaction was determined at 475 nm for 60 min (5 min intervals) at room temperature (25 °C). Results were expressed as a percentage of the control group, after correction to protein content. Five independent experiments were performed.

2.5.4 Determination of acetylcholinesterase (AChE) activity

AChE activity was measured using the method described by Ellman (1961), using acetylthiocholine iodide as a substrate for the homogenates of the flies' heads, using 10 heads of flies per group. The hydrolysis rate of acetylthiocholine iodide was measured at 412 nm through the release of thiol compounds, which reacted with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), producing the colored product thionitrobenzoic acid. Data were expressed as µmol/min/mg protein. Six independent experiments were performed.

2.5.5 Determining levels of reactive species (ROS)

ROS levels were determined through the spectrofluorometric method, according to Pérez-Severiano et al. (2004). Therefore, 20 flies per group were euthanized on ice and the heads were homogenized in 1 mL of 10 mM Tris buffer solution (pH 7.0). Subsequently, the head homogenate was centrifuged at 1.000-x g for 5 min. at 4 °C, and the supernatant was removed and used for quantification of the 2', 7' - dichlorofluorescein diacetate (DCF-DA) oxidation test. The fluorescence emission of the DCF resulting from the oxidation of the DCF-DA was monitored after 1 h with an excitation wavelength of 488 nm and an emission wavelength of 520 nm using a fluorescence spectrometer. Results were expressed as a percentage of the formation of the DCF control in arbitrary units (AU). Six independent experiments were performed.

2.5.6 Thiobarbituric acid reactive substance (TBARS) levels

Lipid peroxidation was estimated through TBARS measurement and was expressed in terms of malondialdehyde (MDA) content, according to the method described by Ohkawa (1979). In this method, MDA, a final product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex. Briefly, the heads of 15 flies from each group were homogenized in a 90 µL HEPES buffer solution (pH 7.0) and centrifuged at 78-x g for 10 min. (4°C). TBARS content was measured in a medium containing 50 µL of tissue homogenate from the head of the flies, 50 µL of 1.2% sodium dodecyl sulfate (SDS), 12 µL of acetic acid buffer (0.45 M, pH 3.4), and 125 µL of thiobarbituric acid (TBA, 8%). The mixture was then heated to 95°C per 120 min in a water bath. After cooling to room temperature, absorbance was measured in the supernatant at 532 nm. The results were calculated as nmol MDA/mg protein. Six independent experiments were performed.

2.5.7 Determination of superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by monitoring the inhibition of quercetin auto-oxidation, according to Kostyuk and Potapovich (1989) with modifications, as described by Franco et al. (2009). The homogenates containing 20 heads of flies from each group were centrifuged at 15.000-x g for 10 min at 4°C. The sodium phosphate buffer reaction mixture (0.025 M/0.1 mM EDTA, pH 10), N, N, N, N-tetramethylethylenediamine (TEMED) was added (1 mL) along with 10 µL of the

sample diluted in 90 µL of a HEPES buffer solution and 50 µL of quercetin, which were monitored at 406 nm for 2 min to assess their autoxidation. They represent the average amount of protein required to inhibit quercetin oxidation by 50% at 26°C. SOD activity was expressed as U/mg protein. Six independent experiments were performed.

2.5.8 Determination of catalase (CAT) activity

CAT activity was tested according to Aebi (1984) with modifications, as suggested by Paula *et al*. (2014). Briefly, homogenates containing 20 heads of flies from each group were centrifuged at 15.000-x g per 10 min at 4°C. CAT activity was estimated by adding 30 µL of the head sample to a reaction mixture containing phosphate buffer (0.25 M/2.5 mM EDTA, pH 7.0), hydrogen peroxide (10 mM, H_2O_2), and Triton X-100 (0.012%). The decomposition of H_2O_2 was monitored at 412 nm for 2 min, and the activity was expressed as U/mg of protein (1U decomposes 1 µmol of H_2O_2/min at pH 7.0 and 25°C). Six independent experiments were performed.

2.5.9 Determination of glutathione S-transferase (GST) activity

To determine GST activity, the method by Habig; Pabst; Jakoby (1974) was followed. Twenty heads of flies were homogenized in 200 µL of a Hepes buffer solution (pH 7.0) for 2 minutes and centrifuged at 15.000-x g for 10 minutes at 4 °C, after which the supernatant fraction was removed. To determine the catalytic action of GST in the conjugation reaction of 1- chloro -2,4-dinitrobenzene (CDNB) with reduced glutathione (GSH) for obtaining a thioether (4-dinitrophenyl glutathione), a spectrophotometer reading was performed at 340 nm for 2 min, using a 1000 µL mixture (0.25 M KPi buffer / 2.5 mM EDTA pH 7.0, 100 mM GSH and distilled water), 30 µL of the sample, and 20 µL CDNB (50 mM) as initiating substrate. The result was expressed as nmol/mg protein. Five independent experiments were performed.

2.6 Statistical analyses

Graph Pad Prism 8 software was used for statistical analyses and plotting graphs. The normality of data was evaluated through the Shapiro-Wilk test. The survival percentage was assessed by comparing survival curves with the log-rank (Mantel-Cox) test. The statistical analysis of the quinoa extract curve was carried out by performing a one-way (ANOVA) followed by the post hoc test by Tukey. The data from the *ex vivo* evaluations were subjected to a two-way ANOVA followed by Tukey's

post hoc for multiple comparisons. Values of $P < 0.05$ were considered statistically significant. All data are expressed as the mean and S.E.M.

3 RESULTS

3.1 Experiment 1

Figure 1 (A-D) shows the experimental protocol, the effect of the exposure to quinoa extract (1.5, 5.0, and 15.0 mg/mL) for 7 days on the survival percentage, climbing time (negative geotaxis test), and crossing number (open field test). The statistical analysis, by comparing the Mantel-Cox log-rank test, showed that the exposure to quinoa extract, regardless of its concentration, did not significantly alter the survival percentage of the flies (Fig. 1B). The one-way analysis of variance (ANOVA) showed that there was no significant difference in the climbing time (Fig. 1C), however, it revealed that the exposure to quinoa extract significantly increased the number of crossings [$F (3, 16) = 3.488$; $P = 0.0405$]. Tukey's *post hoc* test comparisons showed that the quinoa extract at the concentration of 15.0 mg/mL improved locomotor and exploratory activity in the open field test (Fig. 1D), compared to the control group.

3.2 Experiment 2

3.2.1 Behavioral assessment

Figure 2 (A-G) shows the effect of the exposure to rotenone (500 µM) and quinoa extract (5 mg/mL), and the co-exposure to rotenone and quinoa extract for 7 days on the survival percentage, climbing time, crossing number, memory assessment and food consumption of *Drosophila melanogaster*. The statistical analysis, by comparing the Mantel-Cox log-rank test, showed that exposure to rotenone significantly decreased the survival percentage of flies over the experimental period in comparison to the control group. However, quinoa extract co-exposure protected the flies against rotenone-induced mortality ($P = 0.0001$) (Fig. 2B). The two-way ANOVA revealed a significant effect for the interaction factor (quinoa extract versus rotenone) on the climbing time [$F (1, 16) = 18.21$; $P = 0.0006$] and the number of crossings [$F (1, 16) = 11.48$; $P = 0.0038$]. Tukey's *post hoc* test comparisons demonstrated that quinoa extract co-exposure protected the flies against the locomotor and exploratory damage in the geotaxis (Fig. 2C) and open field test (Fig. 2D). The analysis also showed a significant effect for the interaction factor (quinoa extract versus rotenone) on learning

(PC0: immediately after conditioning) [F (1, 68) = 19.48; P = 0.0001)] and short-term memory (PC6: after 6 hours of conditioning) [F (1,68) = 18.40; P = 0.0001)] in the memory assessment. Tukey's *post hoc* test comparisons demonstrated that the exposure to rotenone decreased learning and short-term memory compared to the control group. Co-exposure to quinoa extract protected the flies against impaired learning (Fig. 2E) and (Fig. 2F) short-term memory induced by rotenone. No significant differences were found for the interaction factor (quinoa extract versus rotenone) on the food consumption of the flies (Fig. 2G).

3.2.2 Determination of Dopamine levels, TH and AChE activities

Figure 3 (A-C) shows the effect of the exposure to rotenone (500 µM) and quinoa extract (5 mg/ML) and the co-exposure to rotenone and quinoa extract for 7 days on dopamine levels, and TH and AChE activities in the head of *Drosophila melanogaster*. The statistical analysis (two-way ANOVA) revealed a significant effect for the interaction factor (quinoa extract versus rotenone) on the dopamine levels [F (1, 16) = 15.54; P = 0.0012], and TH [F (1, 16) = 10.79; P = 0.0047)] and AChE [F (1, 20) = 23.20; P = 0.0001)] activities. Tukey's *post hoc* test comparisons demonstrated that quinoa extract co-exposure protected against the decrease in dopamine levels (Fig. 3A), as well as the inhibition of TH (Fig. 3B) and AChE activities (Fig. 3C) induced by rotenone.

3.2.3 Determination of oxidative stress indicators

Figure 4 (A-E) shows the effect of the exposure to rotenone (500 µM) and quinoa extract (5 mg/ML), and the co-exposure to rotenone and quinoa extract for 7 days on oxidative stress indicators (ROS, TBARS, SOD, CAT and GST) in the head of *Drosophila melanogaster*. The statistical analysis (two-way ANOVA) revealed a significant effect for the interaction factor (quinoa extract versus rotenone) on the oxidative stress indicators: ROS [F (1, 20) = 5,281; P = 0.0325)], TBARS [F (1, 20) = 4,965; P = 0.0375)], SOD [F (1, 20) = 18.34; P = 0.0004)], and CAT [F (1, 20) = 5.771; P = 0.0261)]. However, it did not reveal a significant effect on GST enzyme activity (Fig. 4E). Tukey's *post hoc* test comparisons demonstrated that quinoa extract co-exposure protected the flies against ROS production (Fig. 4A), lipid peroxidation increases (Fig. 4B) and decreases in the antioxidant enzymes activity SOD (Fig. 4C) and CAT (Fig. 4D), induced by rotenone.

4 DISCUSSION

In this study, the possible neuroprotective effect of quinoa extract on the PD-like model induced by the exposure of *Drosophila melanogaster* to rotenone in was evaluated. Initially, a concentration-response curve was performed, to evaluate the effect of quinoa extract *per se* in order to find a concentration of quinoa that does not present an effect *per se*. This is extremely important once it avoids masking harmful effects in experimental protocols that evaluate the effect of the extract associated with disease models such as the PD-like model, also avoiding concentrations that may have any toxic effect. Our results showed that quinoa extract (1.5 – 15.0 mg/mL) did not affect the climbing time in the negative geotaxis behavioral test, however, the concentration of 15.0 mg/mL increased the number of crossings in the open field test, compared to the control group. Furthermore, the exposure to quinoa extract did not affect the survival rate. These results show that quinoa extract does not cause toxicity to the flies. Thus, the highest concentration of quinoa extract that did not show effects on the locomotor and exploratory activity was chosen for the experiments that evaluated co-exposure to rotenone.

Exposure to insecticides and pesticides, including rotenone and paraquat, are associated with an increase in the development of PD (SANDERS; GREENAMYRE, 2013; KHATRI; JUVEKAR, 2016). Rotenone is a toxic lipophilic compound that easily crosses the blood-brain barrier and has been frequently used as a chemical model, as it presents pathogenic characteristics of PD, such as mitochondrial dysfunction, leading to the formation of reactive species (GREENAMYRE *et al.*, 2010). Evidence has shown that the exposure or administration of rotenone increases oxidative stress and decreases the activity of antioxidant enzymes and the neurotransmitter dopamine (BETARBET *et al.*, 2000; DE FREITAS COUTO *et al.*, 2019; FERNANDES *et al.*, 2021). Our results support the described effects of rotenone on the neurochemical changes associated with PD. The exposure to rotenone decreased the dopamine levels and the TH and AChE enzymes activities as well as reduced the activities of SOD and CAT enzymes and increased the production of TBARS and ROS. These neurochemical alterations are associated with and probably responsible for the behavioral damage observed in *Drosophila melanogaster*, such as decreased survival rate and locomotor activity, and for causing learning and memory deficits. Surprisingly, the exposure to rotenone did not alter GST activity, an important group of detoxification enzymes that acts against xenobiotics and oxidative stress promoters. The GST

isoenzymes have substrate specificity and carry out several catalytic activities, however, for many species, exposures to foreign compounds can present an adaptive response, becoming a GST substrate (HAYES; PULFORD, 1995; KIKUCHI *et al.*, 1997). According to our results, we suggest that rotenone was not able to change the GST enzyme because the organism probably acquired an adaptive response to the drug. In studies using *Drosophila melanogaster*, rotenone has shown both effects, a decrease (ARAUJO *et al.*, 2015) and an increase (FERNANDES *et al.*, 2021) in GST activity.

In recent years, evidence has shown that phenolic compounds act as a powerful tool in the combat against free radicals and may be a possible treatment for neurodegenerative diseases (GIAMPIERI *et al.*, 2016; AREIZA-MAZO *et al.*, 2018; ORTEGA-ARELLANO; JIMENEZ-DEL-RIO; VELEZ-PARDO, 2019). In the same train of thought, our results show that co-exposure to quinoa extract protected against the neurotoxicity induced by rotenone in the *Drosophila melanogaster* model. Quinoa extract restored SOD and CAT antioxidant enzymes' activities, evidencing a decrease in the levels of TBARS and ROS. The increased oxidative stress is one of the factors involved in dopaminergic neuron degeneration, which results in decreased dopamine availability, the main cause of the development of PD (HE, *et al.*, 2020).

Dopamine is a natural monoamine neurotransmitter that acts by regulating motor functions such as locomotion and movement. Thus, the decrease in dopamine levels is mainly correlated with the motor symptoms of PD (FRISARDI; SANTAMATO; CHEERAN, 2016; HAYES, 2019). Dopamine is synthesized from the decarboxylation of the amino acid tyrosine. Initially, tyrosine is converted to L-dopa, in a reaction catalyzed by the TH enzyme. L-DOPA is then rapidly converted to DA by aromatic L-amino acid decarboxylase (also called DOPA decarboxylase) (KLEIN *et al.*, 2019; NAGATSU *et al.*, 2019), and the TH enzyme that limits the rate of DA synthesis is used as a marker for dopamine production (NAGATSU; NAGATSU, 2016). Dysregulation of TH activity has also been shown to contribute to PD, being essential in the pathogeny of the disease. Associated with decreased oxidative stress, quinoa extract was also able to restore decreased dopamine levels, as well as TH enzyme activity, suggesting protection against rotenone-induced dopaminergic degeneration.

Evidence has described an integration between the dopaminergic and cholinergic systems in PD. In this sense, the decrease in dopamine levels is accompanied by an increase in acetylcholine (ACh) levels, causing an imbalance

between neurotransmitters and hyperactivity of cholinergic mechanisms, which results in the motor and cognitive manifestations observed in PD (YARNALL; ROCHESTER; BURN, 2011). Other studies have shown reduced activity of AChE resulting in increases in the ACh availability in the synaptic cleft in neurodegenerative disorders such as PD (ZHU *et al.*, 2008). AChE is a cholinergic enzyme responsible for the hydrolysis of the neurotransmitter ACh in the synaptic cleft (ARAÚJO; SANTOS; GONSALVES, 2016). Specifically, in *Drosophila melanogaster*, ACh is the most abundant neurotransmitter in the central nervous system (BOPPANA *et al.*, 2017; WHITE *et al.*, 2020). Thus, the decrease in TH activity and dopamine levels accompanied by a decrease in AChE activity must evidence a dopamine/ACh imbalance, which seems to be re-established by the action of quinoa extract. Our results showed that quinoa extract was able to restore the decrease in dopamine levels and TH and AChE activities in *Drosophila melanogaster* exposed to rotenone. Interestingly, the protective effect of quinoa extract on rotenone-induced neurochemical alterations is associated with, and probably responsible for attenuating, the behavioral damage observed in *Drosophila melanogaster*, such as decreased survival rate and locomotor activity, and learning and memory deficits. Corroborating, previous work has shown that the administration of quinoa extract prevented memory deficit and changes in AChE activity and oxidative stress indicators induced by SCP (SOUZA *et al.*, 2020). Additionally, the restoration of cortical AChE activity induced by methylene blue could relieve the rotenone-induced locomotor and memory impairments (ABDEL-SALAM *et al.*, 2014).

The capacity of phenolic compounds present in plants of protecting against damage induced by chemical compounds that generate oxidative stress and that can lead to the development of neurodegenerative diseases is well established in the literature (GIAMPIERI *et al.*, 2016; ORTEGA-ARELLANO; JIMENEZ-DEL-RIO; VELEZ-PARDO, 2019). Quinoa is a pseudocereal with high phenolic-compound content, especially when compared to other pseudocereals (REPO-CARRASCO-VALENCIA *et al.*, 2010). A previous investigation by the group, available in SOUZA *et al* (2020), describes the composition of the quinoa extract (the same one used in the present study), with a predominance of phenolic acids and flavonoids such as p-coumaric acid and hesperidin respectively. The findings of the current study demonstrate the protective effect of quinoa extract against rotenone-induced

neurotoxicity, and we extrapolate that this effect is related to the bioactive compounds it has, which are mainly phenolic compounds.

5 CONCLUSION

In conclusion, our results suggest that quinoa extract attenuated behavioral impairment on survival, locomotor activity, and memory, induced by rotenone. Thus, we believe that the quinoa extract antioxidant capacity is able to combat oxidative stress, restoring dopamine levels and thus re-establishing a possible dopamine/ACh imbalance. The ability to restore neurochemical changes may be associated with improvement in behavioral aspects. These findings contribute to a better understanding of the effects of quinoa extract on the central nervous system, allowing the identification of therapeutic targets that contribute to the treatment of PD symptoms.

Conflict of interest: The authors declare that there are no conflicts of interest.

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Legends

Figure 1. A) Schematic diagram of the experimental protocol for the quinoa extract concentration-response curve. Evaluation of the exposure of *Drosophila melanogaster* to quinoa extract (1.5; 5.0 and 15.0 mg/mL) for 7 days on B) Survival percentage; data were collected every 24 hours for 7 days, and it was determined by comparing the mortality curves of the Mantel-Cox log-rank tests. Data are expressed as mean and SEM, for n = 5 in each group. C) Negative geotaxis and D) Open-field; data are expressed as mean and SEM, for n = 5 in each group. Significance was determined through a one-way analysis of variance (ANOVA), followed by Tukey's test. * Indicates a significant difference ($P<0.05$) compared to the control group.

Figure 2. A) Schematic diagram of the experimental protocol. Effect of the exposure of *Drosophila melanogaster* to rotenone (500 µM), quinoa extract (5.0 mg/mL), and co-exposure to rotenone and quinoa extract for 7 days on B) Survival percentage; data were collected every 24 hours for 7 days, and it was determined by comparing the mortality curves of the Mantel-Cox log-rank tests. Data are expressed as mean and SEM, for n = 5 in each group. C) Negative geotaxis and D) Open-field; data are expressed as mean and SEM, for n = 5 in each group. E) Learning (PC0: immediately after conditioning and F) Short-term memory (PC6: after 6 hours of conditioning); data are expressed as mean and SEM, for n = 18 in each group. G) Food consumption; data are expressed as mean and SEM, for n = 5 in each group. Significance was determined through a two-way analysis of variance (ANOVA), followed by Tukey's test. * Indicates a significant difference ($P < 0.05$) compared to the control group. # Indicates a significant difference ($P < 0.05$) compared to the rotenone group.

Figure 3. Effect of the exposure of *Drosophila melanogaster* to rotenone (500 µM), quinoa extract (5.0 mg/mL), and co-exposure to rotenone and quinoa extract for 7 days on A) dopamine (DA) levels and B) Tyrosine hydroxylase (TH) activity; data are expressed as mean and SEM, for n = 5 in each group. C) AChE activity; data are expressed as mean and SEM, for n = 6 in each group. Significance was determined through a two-way analysis of variance (ANOVA), followed by Tukey's test. * Indicates a significant difference ($P < 0.05$) compared to the control group. # Indicates a significant difference ($P < 0.05$) compared to the rotenone group.

Figure 4. Effect of the exposure of *Drosophila melanogaster* to rotenone (500 µM), quinoa extract (5.0 mg/mL), and co-exposure to rotenone and quinoa extract for 7 days on A) ROS, B) TBARS C) SOD, D) CAT; data are expressed as mean and SEM, for n = 6 in each group. E) GST; data are expressed as mean and SEM, for n = 5 in each group. Significance was determined through a two-way analysis of variance (ANOVA), followed by Tukey's test. * Indicates a significant difference ($P < 0.05$) compared to the control group. # Indicates a significant difference ($P < 0.05$) compared to the rotenone group.

Figures

Figure 1.

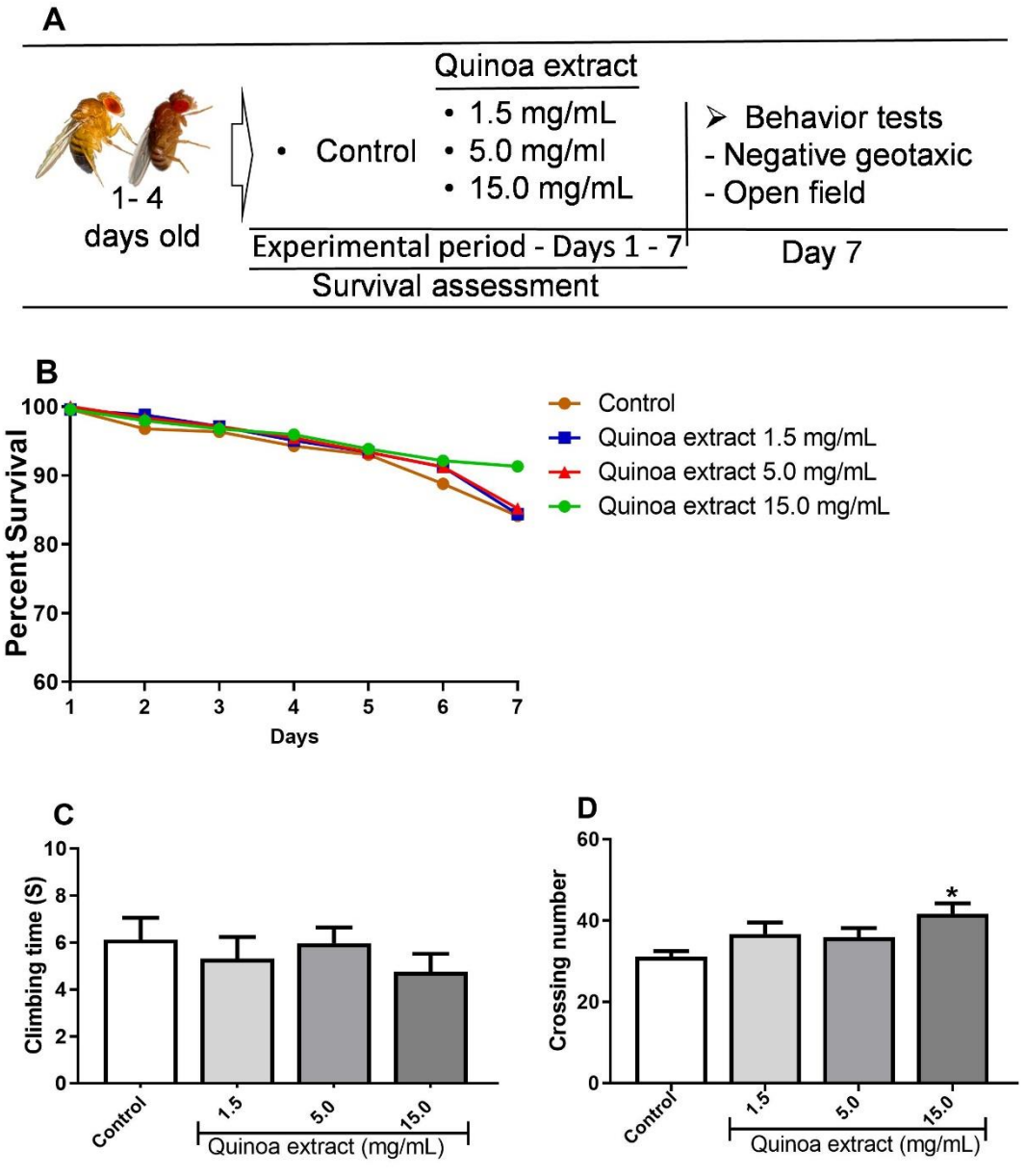


Figure 2.

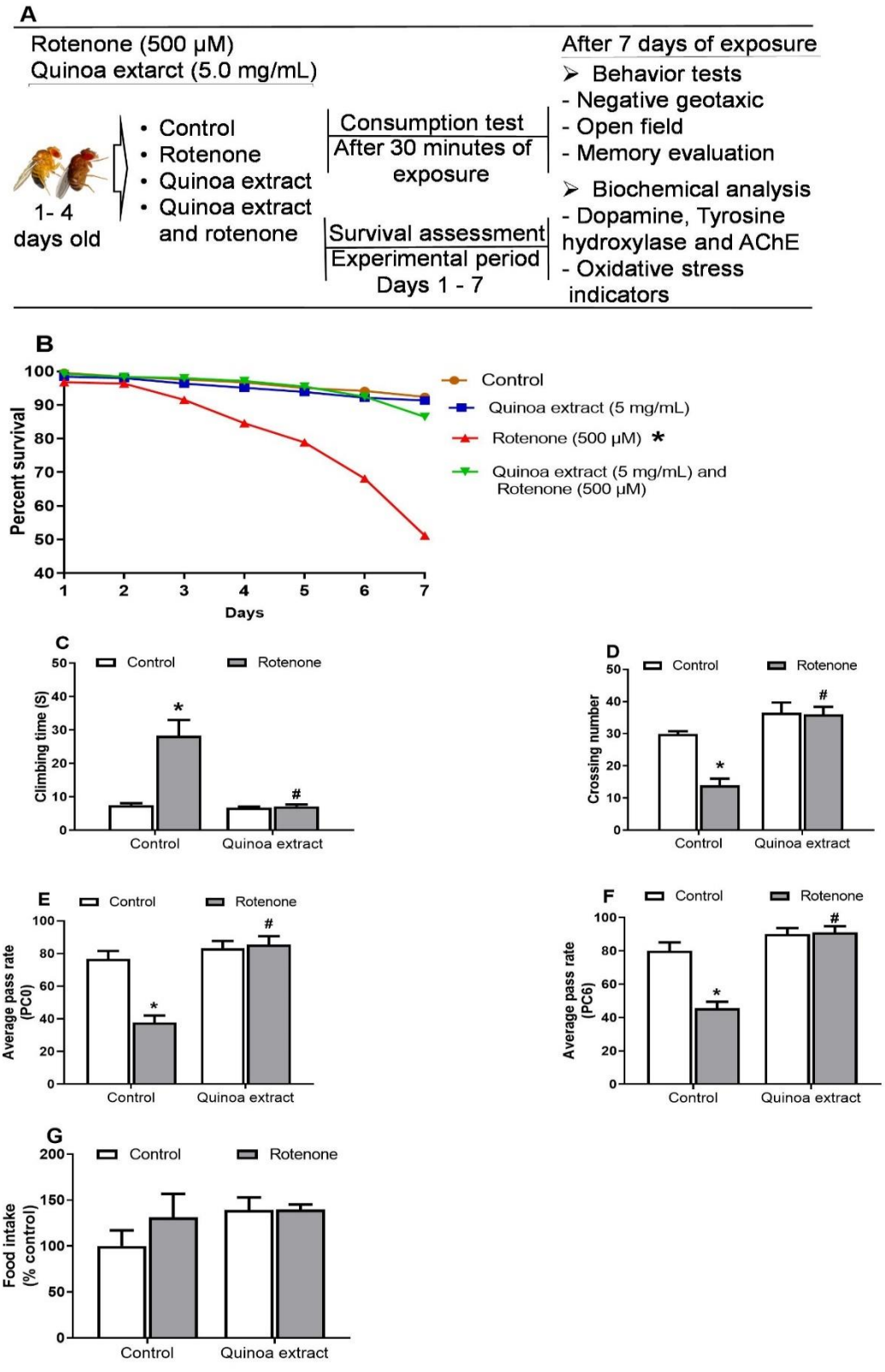

A
Rotenone (500 µM)
Quinoa extarct (5.0 mg/mL)
1- 4 days old
• Control
• Rotenone
• Quinoa extract
• Quinoa extract and rotenone
Consumption test
After 30 minutes of exposure
Survival assessment
Experimental period
Days 1 - 7
After 7 days of exposure
➢ Behavior tests
- Negative geotaxic
- Open field
- Memory evaluation
➢ Biochemical analysis
- Dopamine, Tyrosine hydroxylase and AChE
- Oxidative stress indicators
B
Percent survival
Days
Control
Quinoa extract (5 mg/mL)
Rotenone (500 µM) *
Quinoa extract (5 mg/mL) and Rotenone (500 µM)
C
Climbing time (S)
Control
Rotenone
Control
Quinoa extract
D
Crossing number
E
Average pass rate (PC0)
F
Average pass rate (PC6)
G
Food intake (% control)

Figure 3.

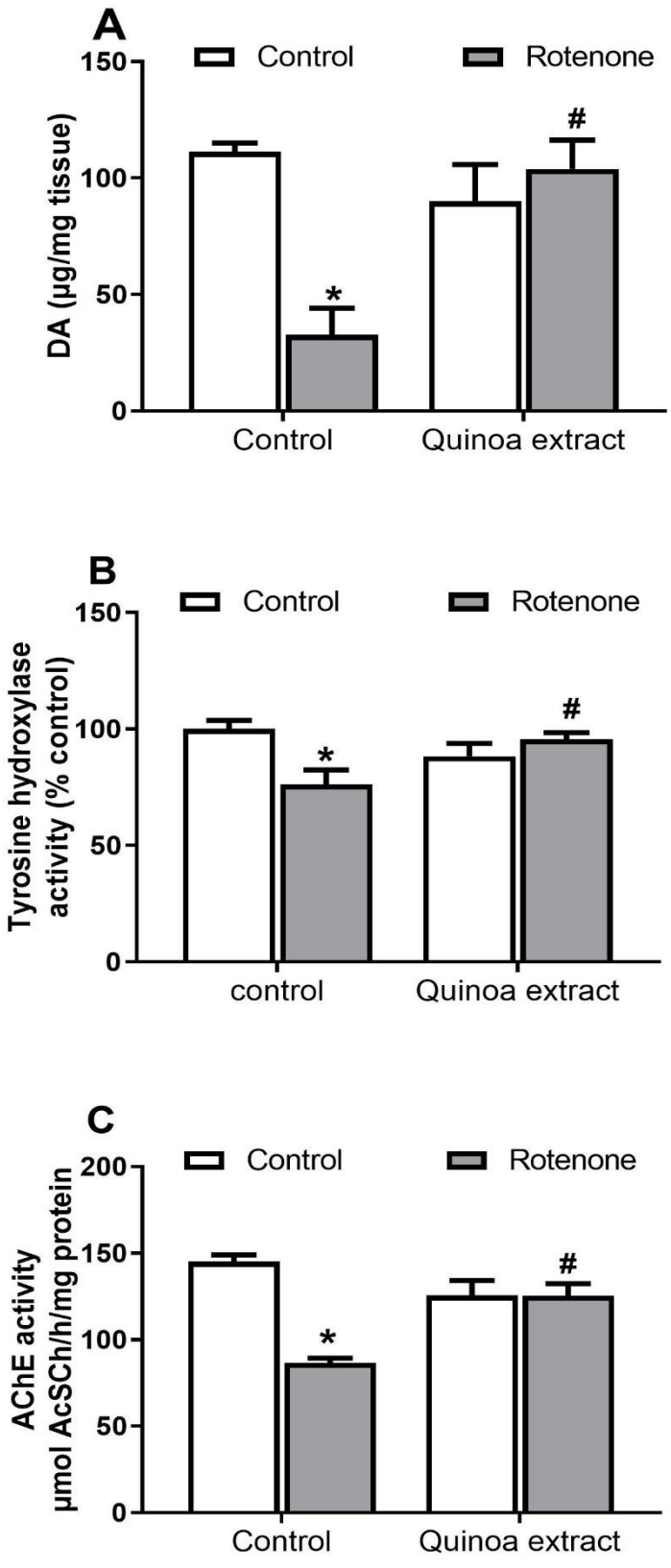
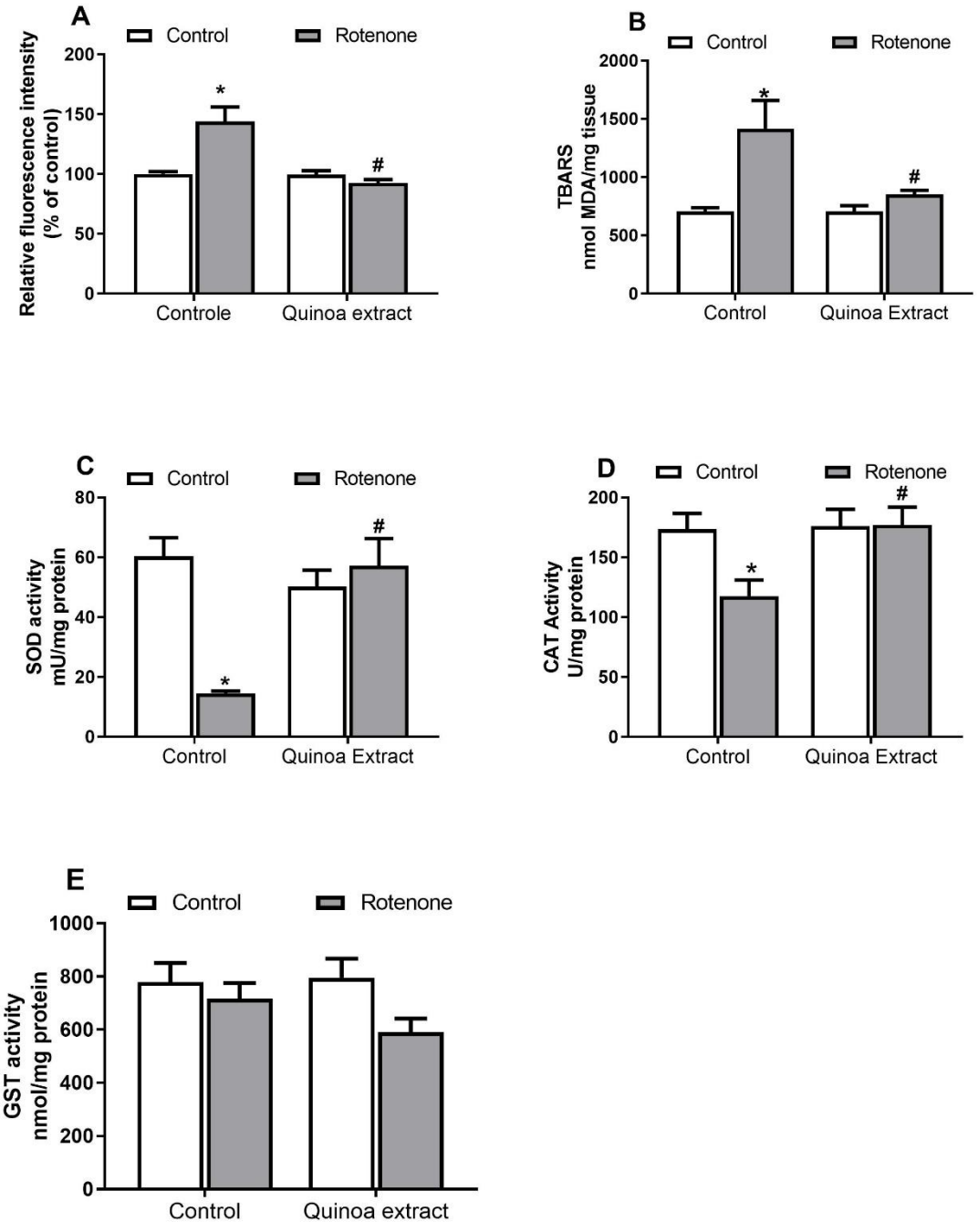

A
Control
Rotenone
DA (µg/mg tissue)
150
100
50
0
*
#
Control
Quinoa extract
B
Control
Rotenone
Tyrosine hydroxylase activity (% control)
150
100
50
0
*
#
control
Quinoa extract
C
Control
Rotenone
AChE activity µmol AcSCh/h/mg protein
200
150
100
50
0
*
#
Control
Quinoa extract

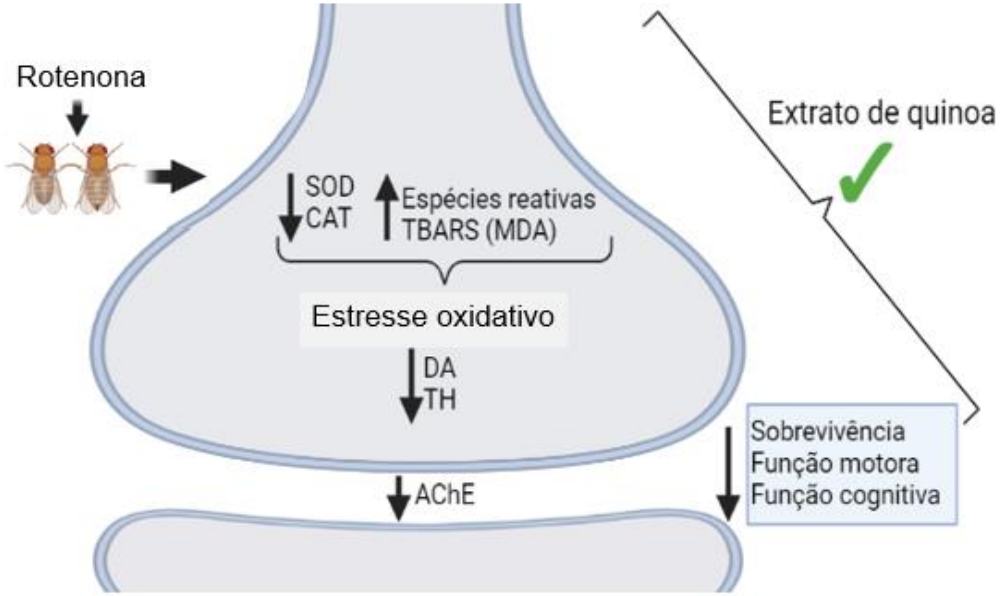
Figure 4.


A
Control
Rotenone
Relative fluorescence intensity (% of control)
200
150
100
50
0
*
#
Controle
Quinoa extract
B
Control
Rotenone
TBARS nmol MDA/mg tissue
2000
1500
1000
500
0
*
#
Control
Quinoa Extract
C
Control
Rotenone
SOD activity mU/mg protein
80
60
40
20
0
*
#
Control
Quinoa Extract
D
Control
Rotenone
CAT Activity U/mg protein
200
150
100
50
0
*
#
Control
Quinoa Extract
E
Control
Rotenone
GST activity nmol/mg protein
1000
800
600
400
200
0
Control
Quinoa extract

5 CONCLUSÃO

Conforme os resultados obtidos neste trabalho, pode-se concluir, que o dano induzido pela rotenona em *Drosophila melanogaster* foram protegidas pela co-exposição ao extrato de quinoa, onde mostra, aumento na taxa de sobrevivência, proteção dos déficits locomotores e de memória, evidenciado pela modulação da dopamina e das enzimas TH e AChE, responsáveis pela síntese da DA e hidrólise da ACh respectivamente, sendo a DA e ACh importantes neurotransmissores responsáveis pelas funções motoras e cognitivas. Bem como, mostrou regulação nos indicadores de estresse oxidativo (Figura 7). Estes resultados sugerem que o extrato de quinoa pode auxiliar no tratamento de doenças neurodegenerativas, como a DP.

Figura 7 - Representação gráfica da conclusão do trabalho



Fonte: Próprio autor

6 PERSPECTIVAS

De acordo com os resultados alcançados consideramos a importância de realizar outros mecanismos envolvidos na doença DP, dentre eles:

- Avaliação de memória a longo prazo;
- Avaliação de memória associado com estímulo de recompensa;
- Determinar a atividade do glutamato;
- Determinar a atividade do ácido gama-aminobutírico (GABA);
- Determinar níveis do neurotransmissor ACh;
- Avaliar a proteína α-sinucleína

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