

**UNIVERSIDADE FEDERAL DO PAMPA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA
CAMPUS URUGUAIANA**

RAFAELA GARAY PIRES

**ANÁLISE DA SEGURANÇA E EFEITO ANTIOXIDANTE DO CAMU-CAMU
(*Myrciaria dubia*) EM *Drosophila melanogaster* EXPOSTAS AO FERRO**

**Uruguaiana
2023**

RAFAELA GARAY PIRES

**ANÁLISE DA SEGURANÇA E EFEITO ANTIOXIDANTE DO CAMU-CAMU
(*Myrciaria dubia*) EM *Drosophila melanogaster* EXPOSTAS AO FERRO**

Dissertação apresentada ao Programa de Pós-graduação
Stricto sensu em Bioquímica da Universidade Federal
do Pampa, como requisito parcial para obtenção do
Título de Mestre em Bioquímica.

Orientadora: Prof^a Dr^a Marina Prigol

Coorientadora: Prof^a Dr^a Fernanda Barbisan

**Uruguaiana
2023**

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) .

P667a Pires, Rafaela Garay
 ANÁLISE DA SEGURANÇA E EFEITO ANTIOXIDANTE DO CAMU-CAMU
 (Myrciaria dubia) EM Drosophila melanogaster EXPOSTAS AO FERRO
 / Rafaela Garay Pires.
 62 p.

 Dissertação (Mestrado)-- Universidade Federal do Pampa,
 MESTRADO EM BIOQUÍMICA, 2023.
 "Orientação: Marina Prigol".

 1. Camu-camu. 2. Drosophila melanogaster. 3. estresse
 oxidativo. 4. bioquímica. I. Título.

Rafaela Garay Pires

**ANÁLISE DA SEGURANÇA E EFEITO ANTIOXIDANTE DO CAMU-
CAMU (*Myrciaria dubia*) EM *Drosophila melanogaster* EXPOSTAS AO FERRO**

Dissertação apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Mestre em Ciências Biológicas II.

Dissertação defendida e aprovada em: 01/08/2023

Banca examinadora:

Prof. Dra. Marina Prigol
Orientador
(UNIPAMPA)

Prof. Dra. Verônica Farina Azzolin
(UFSM)

Prof.Dr. Carlos Borges Filho
(UNIPAMPA)



Assinado eletronicamente por **MARINA PRIGOL, PROFESSOR DO MAGISTERIO SUPERIOR**, em 31/08/2023, às 10:37, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.



Assinado eletronicamente por **Verônica Farina Azzolin, Usuário Externo**, em 04/09/2023, às 10:48, conforme horário oficial de Brasília, de acordo com as normativas legais aplicáveis.



Assinado eletronicamente por **CARLOS BORGES FILHO, Técnico de Laboratório Área**, em 04/09/2023, às 13:51, 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 **1229684** e o código CRC **E0D584FD**.

AGRADECIMENTOS

Primeiramente, gostaria de agradecer a Deus e aos meus guias espirituais por terem me guiado e me auxiliado durante essa caminhada.

Agradeço a minha orientadora Prof. Dr^a. Marina Prigol por ter aceitado me orientar e me auxiliar durante esses dois anos, mesmo morando em outra cidade estava sempre a disposição em me ajudar no que pudesse. Foi uma experiência incrível ser sua orientada e poder aprender muito contigo.

Destaco meu agradecimento a minha coorientadora Prof. Dr^a Fernanda Barbisan, no qual me abriu as portas do Laboratório Biogenômica – UFSM em 2018 quando eu era recém formada e já com o sonho em realizar um mestrado. Sou muito grata pelo que você fez por mim nessa minha trajetória em Santa Maria e mesmo não estando mais no mesmo laboratório e na mesma cidade, foi um alicerce nessa caminhada.

Agradeço aos meus pais Roberto e Mariângela Pires, a minha irmã Fernanda por terem sido a minha base e também por terem me apoiado para chegarem até aqui.

Agradeço ao meu companheiro Luiz Fernando Baldez pela força, companheirismo de muitas noites enquanto eu estudava e sempre tendo uma palavra de apoio para me fortalecer nessa caminhada e chegar até o final. Sou muito grata por te ter em minha vida.

Agradeço imensamente as meninas do Laboratório Laftambio por toda a ajuda e parceria no laboratório durante esses dois anos de experimentos, destacando a doutoranda Elize Musachio pelo auxílio, paciência e disponibilidade em me ajudar em todo o processo da dissertação durante o mestrado, acredito que sem sua prestatividade e flexibilidade em ensinar não conseguiria ter chegado até aqui, agradeço pelo suporte de hoje e sempre.

RESUMO

O fruto amazônico Camu-camu em pó é comercializado como suplemento alimentar e, como todas as substâncias, é fundamental avaliar as dosagens quanto à sua segurança. Para o protocolo experimental, as larvas de *Drosophila melanogaster* serão separadas e divididas em seis grupos: (1) Controle, Camu-camu em pó nas seguintes concentrações: (2) 0,1 mg/mL Camu-camu (3) 0,5 mg/mL Camu-camu (4) 1 mg/mL Camu-camu (5) 5 mg/mL Camu-camu, (6) 10 mg/mL Camu-camu. Expusemos também larvas de segundo estágio a diferentes concentrações de Camu-camu em pó e, após 48h, obtivemos um valor da (concentração letal) CL₅₀ de 4,799 mg/mL. Foi observado que as larvas não foram capazes de se desenvolver completamente nas concentrações acima de 5 mg/mL como evidenciado pela redução na taxa de pupação e eclosão, devido à toxicidade evidenciada por alterações em marcadores de estresse oxidativo, defesas antioxidantes e análise comportamental. Complementar a isso, escolhemos a concentração de 0,5 mg/mL (10X menor que a CL₅₀), por ter demonstrado ser segura no primeiro momento desse trabalho, e realizamos análises para demonstrar sua ação protetora contra o efeito oxidativo do ferro (Fe). Foi verificado que a produção de espécies reativas e a peroxidação lipídica foram preservadas pelo Camu-camu a nível de controle quando as moscas expostas ao Fe, juntamente com a atividade das enzimas superóxido dismutase (SOD), catalase (CAT) e glutathione-s-transferase (GST). O extrato de Camu-camu também minimizou a possível mortalidade de moscas quando expostas ao Fe, preservou a viabilidade celular e protegeu as moscas de desenvolver alterações no comportamento e atividade da acetilcolinesterase (AChE). O extrato de Camu-camu tornou o organismo de *Drosophila melanogaster* resistente aos insultos oxidativos gerados pela exposição ao Fe, demonstrando potencial terapêutico e de tratamento preventivo, sugerindo que seu consumo durante o desenvolvimento, em concentrações adequadas, pode desenvolver maior resistência a fatores pró-oxidantes.

Palavras-chave: Camu-camu; *Drosophila melanogaster*; estresse oxidativo; morfologia, bioquímico

ABSTRACT

The Amazonian fruit Camu-camu powder is marketed as a food supplement and, like all substances, it is fundamental to evaluate the dosages regarding its safety. For the experimental protocol, the *Drosophila melanogaster* larvae will be separated and divided into six groups: (1) Control and Camu-camu powder at the following concentrations: (2) 0.1 mg/mL Camu-camu (3) 0.5 mg/mL Camu-camu (4) 1 mg/mL Camu-camu (5) 5 mg/mL Camu-camu, (6) 10 mg/mL Camu-camu. We also exposed second-stage larvae to different concentrations of Camu-camu powder and, after 48h, we obtained a CL50 (lethal concentration) value of 4.799 mg/mL. It was observed that the larvae were not able to fully develop at concentrations above 5 mg/mL as evidenced by the reduction in the rate of pupation and hatching, due to toxicity evidenced by alterations in markers of oxidative stress, antioxidant defenses and behavioral analysis. Complementing this, we chose the concentration of 0.5 mg/mL (10X lower than the LC50), as it was shown to be safe in the first moment of this work, and we performed analyzes to demonstrate its protective action against the oxidative effect of iron (Fe). It was verified that the production of reactive species and lipid peroxidation were preserved by Camu-camu at the control level when the flies were exposed to Fe, together with the activity of the enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione-s- transferase (GST). Camu-camu powder also minimized the possible mortality of flies when exposed to Fe, preserved cell viability and protected flies from developing changes in behavior and acetylcholinesterase (AChE) activity. Camu-camu powder made the *Drosophila melanogaster* organism resistant to oxidative insults generated by exposure to Fe, demonstrating therapeutic and preventive treatment potential, suggesting that its consumption during development, in adequate concentrations, may develop greater resistance to pro-oxidants.

Key-words: Camu-camu; *Drosophila melanogaster*; oxidative stress; morphology, biochemistry

LISTA DE FIGURAS

Referencial teórico

Figura 1. Os 7 pilares do envelhecimento.	3
Figura 2. Sistema antioxidante e eliminação do peróxido de hidrogênio (H ₂ O ₂)	4
Figura 3. Imagem do fruto <i>Myrciaria dubia</i> (Camu-camu).	8

Manuscrito

Figure 1. Experimental protocol with Camu-camu powder	19
Figure 2. Schematic representation of the experimental design.	21
Figure 3. Evaluation of the lethal concentration of Camu-camu that killed 50% (LC50) of exposed larvae.	26
Figure 4. Evaluation of pupation (A) and hatching (B) of <i>Drosophila melanogaster</i> exposed to different concentrations of Camu-camu powder.	27
Figure 5. Evaluation of the behavior of <i>Drosophila melanogaster</i> exposed during development to different concentrations of Camu-camu. (A) open-field test and (B) negative geotaxis.....	28
Figure 6. Evaluation of the behavior of <i>Drosophila melanogaster</i> exposed during development to different concentrations of Camu-camu. A) Reactive Species and B) reactive species to thiobarbituric acid.....	29
Figure 7. Evaluation of the activity of the enzymes (A) superoxide dismutase (SOD), (B) catalase (CAT) and (C) Glutathione-S-transferase (GST) in <i>Drosophila melanogaster</i> exposed to different concentrations of Camu-camu powder.	30
Figure 8. Evaluation of gene expression by analysis and rt-PCR of the enzymes (A) Superoxide dismutase (SOD), (B) Catalase (CAT) and (C) Cytochrome P-450 (CYP450), in flies exposed during development to different concentrations Camu-camu powder.	31
Figure 9. Evaluation of Acetylcholinesterase (AChE) enzyme activity in <i>Drosophila melanogaster</i> exposed to different concentrations of Camu-camu powder.	32
Figure 10. Evaluation of cell viability by the resazurin reduction method in <i>Drosophila melanogaster</i> exposed to different concentrations of Camu-camu powder.	33
Figure 11. Evaluation of mortality, in 24h of exposure to Iron (Fe), of flies that consumed Camu-camu during development.....	34
Figure 12. Evaluation of behavioral tests in flies exposed during development to Camu-camu against exposure to Iron (Fe) as adults.	35
Figure 13. Quantification of (A) reactive species (RS) and (B) lipid peroxidation by quantification of thiobarbituric acid reactive species (TBARS) in flies exposed during development to Camu-camu versus exposure to Iron (Fe) as adults.....	36
Figure 14. Evaluation of the activity of (A) superoxide dismutase (SOD), (B) catalase (CAT) and (C) glutathione-S-transferase (GST) enzymes in flies exposed during development to Camu-camu (CC) versus exposure to Iron (Fe) as adults.....	37
Figure 15. Evaluation of the enzyme Acetylcholinesterase (AChE) activity in flies exposed during development to Camu-camu versus exposure to Iron (Fe) as adults.	38
Figure 16. Evaluation of cell viability by the resazurin reduction method in flies exposed during development to Camu-camu versus exposure to Iron (Fe) as adults.....	39

LISTA DE TABELAS

Referencial teórico

Tabela 1. Resumo dos padrões alimentares comuns.	6
Tabela 2. Compostos bioativos promissores relacionados às biofuncionalidades do fruto do Camu-camu (<i>Myrciaria dubia</i>).....	9
Tabela 3. Análise HPLC–DAD–ESI-MS–MS de compostos fenólicos de Camu-camu em pó.	10

Manuscrito

Table 1. Nutritional value in 5g of Camu-camu powder.....	20
Table 2. Gene primers list.....	25

SUMÁRIO

LISTA DE FIGURAS	8
LISTA DE TABELAS	9
1. INTRODUÇÃO.....	1
2. REVISÃO DA LITERATURA	2
2.1- Envelhecimento e Doenças Crônicas	2
2.2- Papel do Estresse Oxidativo no desenvolvimento de doenças Crônicas.....	3
2.3-Papel da Dieta na Prevenção das Doenças	5
2.4 Dieta Amazônica	7
2.4.1. Camu-camu: Características Botânicas e uso popular.....	7
2.4.2 Camu-camu: Compostos Bioativos	8
2.5.2 Camu-camu: Propriedades Biológicas	9
2.6 <i>Drosophila melanogaster</i> como modelo animal para estudos de segurança.....	12
3. OBJETIVOS.....	14
3.1 Objetivo geral:	14
3.2 Objetivos específicos:.....	14
4. RESULTADOS (apresentados na forma de manuscrito)	14
1. INTRODUCTION	17
2. MATERIALS AND METHODS	18
2.1 <i>Drosophila melanogaster</i> stok	18
2.2 Experimental protocol with Camu-camu powder.....	19
2.3 Lethal concentration (LC50)	20
2.4 Exposure treatment with Iron	20
2.5 Pupation and hatching rate	21
2.6 Behavioral tests	21
2.7 Quantification of reactive species.....	22
2.8 Evaluation of lipid peroxidation by the method of quantification of reactive species to thiobarbituric acid.....	23
2.9 Superoxide dismutase, catalase and glutathione-s-transferase activity	23
2.10 Protein quantification	24
2.11 Gene Expression by qRT-PCR.....	24
2.12 Statistical analysis	25
3. RESULTS.....	26
4. DISCUSSION.....	39
5. CONCLUSION	43

6. REFERENCES	44
5. CONSIDERAÇÕES FINAIS	47
6. REFERÊNCIAS	48

1. INTRODUÇÃO

Uma dieta rica em frutas e vegetais tem se mostrado benéfica para a prevenção de inúmeras doenças crônicas não transmissíveis (Shahidi e Ambigaipalan, 2015). Neste contexto e levando em conta a biodiversidade de plantas da Amazônia Brasileira nos oferecem um grande potencial terapêutico atribuído ao conteúdo de fitoquímicos bioativos (Sosa et al., 2019). Dentre os vários frutos amazônicos destacamos a *Myrciaria dubia* (Camu-camu), esse fruto que também é conhecido como caçari, é um fruto de uma árvore típica da Amazônia, que cresce nas margens de rios, córregos, lagos e mangues. Este é um fruto consumido pela população local na forma de geleias, sorvetes e licores, uma vez que é considerado ácido para consumo in natura (Cavalieri, 1993; Franco e Janzantti, 2005). O interesse no consumo do Camu-camu têm aumentado na população por conta da sua quantidade alta de vitamina C, e por conta disso, esse composto “promete” auxiliar na redução de possíveis doenças crônicas não-transmissíveis no qual são resultantes do excesso de produção de espécies reativas de oxigênio no organismo in vivo.

Estudos científicos indicam que o Camu-camu apresenta fitoquímicos que estão associados a várias propriedades importantes tais como antioxidante e antiinflamatório (Castro et al., 2018 ; Sosa et al., 2018), e como não há nenhuma contra-indicação da ingestão do Camu-camu em pó e o mesmo está sendo comercializado a livre demanda para a sociedade, se verifica a necessidade de um estudo que investiga concentrações diferentes do extrato de Camu-camu, ou seja, se observa a indispensabilidade de analisarmos a segurança deste suplemento alimentar para assim, visarmos seus possíveis decorrências no organismo *in vivo*. E no mesmo argumento, se leva em consideração também em realizar uma curva de concentração resposta para reforçar qual concentração é considerada segura desse fruto.

A partir deste argumento, levamos em consideração a necessidade de usar um modelo pró oxidante para analisar se o efeito do fruto Camu-camu evidencia essa eficiência protetora e antioxidante para o organismo. Para isso, em estudos científicos têm se apresentado o Ferro (Fe) como modelo de exposição pró oxidante. Esclarecendo o uso de Fe para esse estudo, o mesmo é um metal de transição e metal redox-ativo, no qual contribui para a interação intensificada com o oxigênio (O₂) que eventualmente dá origem à geração de espécies reativas de oxigênio (ROS), no qual podem ser deletérias para os organismos em circunstâncias específicas e participar da deterioração celular em vários estágios, dentre elas: ácido desoxirribonucleico (DNA), proteína (α -sinucleína) e membrana lipídios (glicolipídios,

fosfolipídios e colesterol) (Behl et al., 2022). Sendo assim, confirmando ser uma referência padrão para avaliarmos nesta pesquisa a eficácia antioxidante deste fruto amazônico. Assim, neste estudo nos propomos a avaliar a segurança do consumo do Camu-camu bem como verificar seus efeitos antioxidantes.

2. REVISÃO DA LITERATURA

2.1- Envelhecimento e Doenças Crônicas

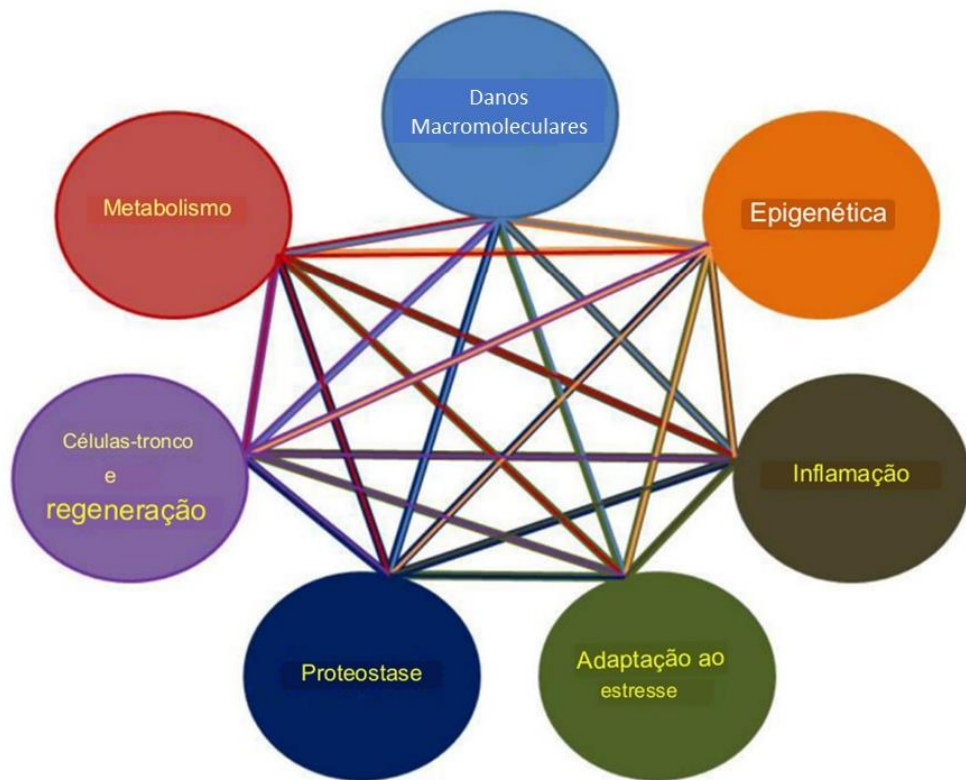
De acordo com o estudo de Dominguez e colaboradores (2021) a expectativa média de vida da população mundial aumentou notavelmente nos últimos 150 anos, e no momento atual muitos cidadãos da maioria dos países europeus e do Japão vivem mais de 80 anos.

O envelhecimento é caracterizado pelo declínio funcional progressivo a níveis moleculares, celulares, teciduais e do organismo. É o acúmulo progressivo de mudanças com o tempo que estão associadas ou são responsáveis pela suscetibilidade cada vez maior à doença e à morte que acompanha o avanço da idade, é um processo fisiológico contínuo e independente do gênero. Essas mudanças relacionadas ao tempo são atribuídas ao processo de envelhecimento, no qual apresenta interações entre fatores genéticos (genoma) e ambientais (epigenoma), que operam durante toda a vida de uma pessoa e estão associadas a uma diminuição ou redução da capacidade de recuperação de danos físicos ou mutagênicos que aumentarão concomitantemente o risco de doenças. (Harman, 1981 ; Chedraui e Pérez-López, 2013 ; Booth e Brunet, 2016).

De acordo com Kennedy e colaboradores (2014), as doenças crônicas constituem a maior parte da carga global de doenças e são as causas mais comuns de mortalidade. A investigação médica tem historicamente visado as doenças separadamente, assumindo que os sistemas orgânicos atuam de forma independente. No entanto, a funcionalidade ideal para qualquer sistema único requer a saúde geral do organismo, impelindo investigações sobre a interdependência destas relações para desenvolver modelos de rede em múltiplas escalas que incorporem as mudanças fisiológicas que acompanham o envelhecimento. Visar doenças individualmente para uma população idosa também é complicado porque a maioria dos idosos tem múltiplas morbidades que interagem, confundindo as estratégias terapêuticas. Ao compreender como o envelhecimento permite a patologia, surgirão novas terapêuticas para

múltiplas doenças crônicas, proporcionando uma oportunidade de prolongar o tempo de saúde humana, visando diretamente o envelhecimento. Na figura 1 citam os 7 pilares do envelhecimento de acordo com o mesmo estudo.

Figura 1. Os 7 pilares do envelhecimento.



FONTE: Kennedy et al. (2014) adaptado pelo autor.

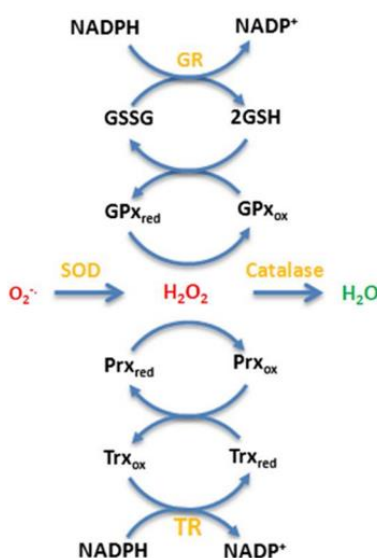
A teoria dos radicais livres do envelhecimento, posteriormente denominada como teoria do estresse oxidativo do envelhecimento, baseia-se na hipótese baseada em danos estruturais de que as perdas funcionais associadas à idade são devidas ao acúmulo de danos oxidativos em macromoléculas (Liguori, 2018).

2.2- Papel do Estresse Oxidativo no desenvolvimento de doenças Crônicas

O termo “estresse oxidativo” é provavelmente um dos termos mais vagos e frequentemente abusados na ciência, perdendo apenas para o termo “inflamação”. O conceito de estresse oxidativo foi introduzido para pesquisas em biologia redox e medicina em 1985. Uma revisão intitulada 'Bioquímica de Estresse Oxidativo' apresentou o conhecimento sobre

pró-oxidantes e antioxidantes e suas fontes endógenas e exógenas e sumidouros metabólicos (Day, B. 2014 e Sies, H. 2015). Ainda no estudo de Day (2014) cita que os sistemas antioxidantes que regulam os níveis celulares de H_2O_2 também dependem de NADPH. Ambos os sistemas de glutathiona peroxidase (GPx) e peroxirredoxina (Prx) dependem de glutathiona (GSH) e tiorredoxina (Trx), respectivamente, para eliminar H_2O_2 e seus produtos oxidados são reciclados pelas glutathiona e tiorredoxina redutases que utilizam NADPH, respectivamente (Figura 2).

Figura 2. Sistema antioxidante e eliminação do peróxido de hidrogênio (H_2O_2)



FONTE: DAY et al., 2014

Em sua grande parte dos tipos de células, as mitocôndrias são os principais impulsionadores da produção de oxidante intracelular, enquanto outras fontes relevantes são as oxidases de nicotinamida adenina dinucleotídeo fosfato (NADPH). Além disso, inúmeras outras enzimas, como xantina oxidase, óxido nítrico sintase, ciclooxygenases, enzimas do citocromo P450 e lipoxigenases, tanto quanto outras organelas celulares, no qual contribuem para a produção de ROS intracelular (Senoner e Wolfgang, 2019).

Em alguns estudos pode-se demonstrar que o estresse oxidativo participa de uma ampla diversidade de doenças crônicas não transmissíveis, incluindo aterosclerose, doença pulmonar obstrutiva crônica (DPOC), doença de Alzheimer e câncer, o que revelou os múltiplos mecanismos pelos quais os oxidantes apresentam um papel importante no dano celular. Entretanto, a extensão em que o estresse oxidativo participa na patologia das doenças tem uma

ampla variedade, de modo que a eficácia do aumento da defesa antioxidante pode ser limitada dependendo da doença apresentada (Forman, H. J.; Zhang, H., 2021). Outros danos causados pelo alto estresse oxidativo não fisiológico levam a uma ampla gama de alterações fenotípicas, incluindo expressão gênica alterada, proliferação e crescimento celular interrompidos e senescência celular (Luo et al., 2019).

Na pesquisa de Tan, Norhaizan e Liew (2018) estudos apontam e exploram a influência de um padrão alimentar ocidentalizado em doenças inflamatórias, como o câncer colorretal, mostraram consistentemente uma tendência semelhante. Tais achados destacam a ideia fundamental de que a qualidade da alimentação pode afetar a função imunológica e a inflamação sistêmica do organismo humano.

2.3 Papel da Dieta na Prevenção das Doenças

De acordo com a pesquisa de Ozemek e contribuintes (2018) o consumo regular de uma dieta rica em sódio, alimentos densos em energia, gordura, carboidratos refinados, adição de açúcar e pobre em frutas e vegetais contribui para um risco aumentado de desenvolver doenças crônicas não transmissíveis, como hipertensão (HAS) e doenças cardiovasculares.

Pode-se dizer que há vários tipos de dietas que possam ser utilizadas para prevenção de doenças, dentre uma delas é a dieta mediterrânea (MedDiet), no qual refere-se ao padrão alimentar tradicional característico de populações ribeirinhas do Mar Mediterrâneo nos anos sessenta. Ela é caracterizada por uma proporção relativamente alta de frutas, vegetais, legumes e cereais, carne branca e peixe como fonte primária de proteína e azeite de oliva como principal fonte de gordura, então identificada como uma dieta com composição potencialmente saudável (Salas-Salvadó et al., 2018; Delgado-Lista et al., 2022). Miranda, Gómez-Gaete e Mennickent (2017) destacam em sua pesquisa a dieta mediterrânea como uma prevenção para a doença de Alzheimer, pois a mesma apresenta consumo de alimentos que fornecem ácidos graxos ômega 3 estão associados com a diminuição do risco de demência e declínio cognitivo, devido à incorporação de ácido eicosapentaenóico (EPA) e ácido docosahexaenóico (DHA) através da dieta, que têm efeitos anti-inflamatórios e são essenciais para o bom funcionamento neuronal e cerebral.

Pesquisa demonstra os benefícios de dietas à base de vegetais no tratamento do diabetes tipo 2 e na redução das principais complicações macrovasculares e microvasculares

relacionadas ao mesmo. As evidências sugerem que o tipo e a fonte de carboidratos (não refinados versus refinados), gorduras (monoinsaturadas e poliinsaturadas versus saturadas e trans) e proteínas (vegetais versus animais) desempenham um papel importante na prevenção e controle da diabetes tipo 2. Múltiplos mecanismos potenciais fundamentam os benefícios de uma dieta baseada em vegetais na melhoria da resistência à insulina, incluindo promoção de um peso corporal saudável, aumento de fibras e fitonutrientes, interações alimento-microbioma e diminuição de gordura saturada, produtos finais de glicação avançada, nitrosaminas e heme ferro (McMacken e Shah, 2017).

Locke, Schneiderhan e Zick (2018) citam que recomendar um estilo alimentar pode ser uma forma de apoiar mudanças alimentares saudáveis. A lista de dietas populares é longa e a maioria não foi avaliada cuidadosamente. Em destaque, a dieta mediterrânea tem evidências moderadas a fortes de prevenção do diabetes mellitus tipo 2, diminuição da incidência e mortalidade por câncer, prevenção do declínio cognitivo relacionado à idade, prevenção da incidência e mortalidade de doenças cardiovasculares (DCV), diminuição da mortalidade geral e tratamento da obesidade. As mesmas citam que os açúcares adicionados na dieta devem ser limitados a menos de 5% a 10% da ingestão calórica diária. Legumes (não incluindo batatas) e frutas devem constituir metade de cada refeição. As fontes de carboidratos devem incluir principalmente feijões/leguminosas, grãos integrais, frutas e vegetais (Tabela 1).

Tabela 1. Resumo dos padrões alimentares comuns.

Dieta	Inclui	Restrições	Benefícios para a saúde	Considerações especiais
Abordagens dietéticas para reduzir a Hipertensão	52% a 55% de carboidratos, 16% a 18% de proteínas e 30% de gorduras totais; rico em frutas, vegetais, grãos integrais e laticínios com baixo teor de gordura.	Limita gorduras saturadas, colesterol, grãos refinados e açúcares; a ingestão sugerida de sódio é inferior a 2.400mg por dia.	Diminui os fatores de risco de doenças cardiovasculares (DCV), pressão arterial, obesidade e diabetes mellitus tipo 2.	---
Mediterrâneo	Peixes, gorduras monoinsaturadas do azeite, frutas, vegetais, grãos integrais, legumes/nozes e consumo moderado de álcool.	Limita carne vermelha, grãos refinados e açúcares.	Diminui a taxa de diabetes mellitus tipo 2, incidência e mortalidade por câncer, declínio cognitivo relacionado a idade, incidência e mortalidade por DCV, mortalidade geral e obesidade.	---
Vegetariano ou Vegano	Alimentos a base de plantas, grãos, óleos vegetais, nozes, sementes, frutas e vegetais.	Dieta vegetariana: evita carne vermelha, porco, aves, peixes e possivelmente ovos; Dieta vegana: exclui todos os produtos de	Dieta vegetariana diminui taxas de diabetes tipo 2 e doença arterial coronariana; Dieta vegana diminui taxas de hipertensão, obesidade e mortalidade por DCV	A preocupação com as deficiências de vitaminas nas dietas veganas, especialmente a suplementação de vitamina B ₁₂ pode ser

origem animal e, em alguns casos, mel.	necessária; Pessoas que escolhem uma dieta vegetariana devem certificar-se de comer alimentos de todos os grupos alimentares.
--	---

FONTE: Locke, Schneiderhan e Zick (2018) adaptado pelo autor.

2.4 Dieta Amazônica

Já se tem o conhecimento que as boas práticas alimentares, através de uma dieta adequada rica em frutas e verduras, podem possuir um grande número de efeitos benéficos à saúde humana, devido especialmente aos efeitos antioxidante, anti-inflamatório, analgésico e antitumoral de vários desses alimentos (Cold, 1986; Prior e Cao, 2000).

A dieta amazônica além de ser composta por uma grande variedade de frutos e ervas únicas da região (Wyatt et al., 2017), contém inúmeros alimentos ricos em nutrientes e minerais essenciais para a saúde e são interessantes por vários motivos que vão além da proteína animal. A maioria das dietas na amazônia são baseadas nas produções locais dos alimentos, como por exemplo: a horticultura, pesca, caça e atividades de coleta (Dufour et al., 2016).

De acordo com o estudo de Maciel e colaboradores (2021) tradicionalmente os povos indígenas dedicam a maior parte de seu tempo em atividades voltadas para a alimentação. O plantio, a colheita, a caça e a pesca são atividades diárias que embasam a alimentação do grupo, de acordo com a região em que eles vivem.

No Brasil, alguns estudos baseiam-se no fato da grande biodiversidade vegetal encontrada principalmente na Amazônia, no qual contém mais de 30.000 espécies de plantas e alimentos ricos em compostos bioativos como terpenos, ácidos fenólicos, alcaloides e flavonoides, com potenciais benefícios à saúde (antioxidante, antimicrobiano, antiparasitário, anticancerígeno, gastroprotetor, prebiótico, entre outros) e que os mesmos necessitam de maiores análises e definições (Lima, Carvalho e Conte-Júnior, 2022). E dentre essa diversidade alimentar riquíssima na Amazônia, têm se destacado várias frutas que chamam a atenção da população em busca de uma vida mais saudável, no qual são chamadas de “superfrutas”, dentre elas está o fruto Camu-camu.

2.4.1. Camu-camu: Características Botânicas e uso popular

Myrciaria dubia - “camu camu” (conforme a figura 3) é um arbusto frutífero nativo da Amazônia que se desenvolve em áreas expostas a inundações frequentes nas margens de rios, córregos, lagos e pântanos de vários países amazônicos, distribuída principalmente nas florestas

amazônicas do Peru, Brasil, Venezuela e Colômbia. Esta espécie de planta possui um genoma diplóide, e o tamanho do seu genoma (230 Mb) está na faixa de outras espécies de *Myrtaceae* (Castro et al., 2018) e nela contém frações de extratos hidrometanólicos e hidroacetônicos, e em seu extrato bruto da semente e casca, apresentaram atividade antioxidante devido aos seus glicosídeos C-elagitanino. E além disso, a polpa possui altos teores de ácido ascórbico (AA) (Neri-Numa et al., 2018; Pardo-Aldave, K., Pareja-Vásquez, M., Guillén, A., 2019).

A fruta não é consumida em seu estado natural, exceto pelos povos indígenas que habitam os territórios naturais da fruta, devido à sua altíssima acidez (Nascimento et al., 2013; Do et al., 2022; Langley et al., 2015).

Figura 3. Imagem do fruto *Myrciaria dubia* (Camu-camu).



FONTE: <<https://ainfo.cnptia.embrapa.br/digital/bitstream/item/175155/1/COLECAO-PLANTAR-A-cultura-do-camu-camu-2012.pdf>>

2.4.2 Camu-camu: Compostos Bioativos

O interesse por esta fruta tem aumentado devido à quantidade impressionante de vitamina C, que pode chegar a 6 g/100 g de polpa fresca. Isso o torna uma das fontes mais ricas de vitamina C do mundo, e também contém níveis significativos de: potássio, cálcio, vitamina A, glicose, frutose, amido, pectina, fósforo e nitrogênio, e também o Camu-camu é consumido globalmente em bebidas, alimentos funcionais e cosméticos em diferentes formas de polpa congelada, suco e extrato. Além da composição química variada, os subprodutos do camu-camu, como as sementes, têm sido associados a bioatividades *in vitro* e *in vivo*, como

antioxidante, antimutagênico, citotóxico, anti-inflamatório, antimicrobiana, anti-hipertensiva e neuroprotetora. Além disso, sua semente tem se mostrado com uma alta quantidade de teor fenólico, se mostrando assim como um grande aliado antimalárico e antiesquistossomicida (Do Carmo et al., 2020).

Sabe-se que o consumo de frutas e hortaliças, ricas em vitamina C, carotenoides e/ou polifenóis, diminui o risco de doenças por atuarem como antioxidantes naturais. Por isso, o perfil químico de compostos bioativos contidos em algumas frutas tropicais, como o Camu-camu, tem levado à pesquisa de fontes naturais para tratar e prevenir algumas doenças metabólicas em substituição ao tratamento medicamentoso. A partir disso, foi verificado que o Camu-camu tem altas propriedades benéficas para a saúde que potencialmente podem prevenir algumas doenças crônicas não transmissíveis, como diabetes tipo 2, síndrome metabólica, obesidade e hipertensão, e condições relacionadas ao estresse oxidativo como inflamações, e outros (García-Chacón, Marín-Loaiza e Osorio, 2023).

Tabela 2. Compostos bioativos promissores relacionados às biofuncionalidades do fruto do Camu-camu (*Myrciaria dubia*).

	Atividade antioxidant e	Atividade anti- hiperglicê mica	Atividade anti- hipertensiva	Atividade anti- obesidade	Reduzir lesões hepáticas e progressão de tumor	Antimicrobiano, anti-hemolítico e anti-leishmania
Compostos bioativos	Casuarinina	Casuarictina	Ácido elágico	Quercetina	1- metilmalato	Metilvescalagina
	Ácido ascórbico	Ácido felúrico	Ácido cumárico	Rutina	Castalaginina	Ácido 2,4- dihidroxibenzóico
	Grandinina	Ácido serínico	Ácido rosmarínico	Miricetina		

FONTE: García-Chacón, Marín-Loaiza e Osorio (2023) adaptado pelo autor.

2.5.2 Camu-camu: Propriedades Biológicas

Estudos tem mostrado seu efeito protetor, como seu efeito sinérgico na antimutagênese nas suas primeiras etapas de mutação, sendo assim protegendo as células de lesões primárias no DNA (Azevedo et al., 2019). No estudo de Do e colaboradores (2022) foi analisado os efeitos antialérgicos da fruta no qual foi extraído por inibição dos receptores de

histamina H1 e H4 e histidina descarboxilase em células RBL-2H3, neste mesmo pode-se observar um resultado positivo do uso da fruta para este fim, e que também os pesquisadores salientam as funções multi-alvo do extrato de Camu-camu e sugerem que ele pode ser um substituto para drogas sintéticas comerciais para contribuir como um candidato eficaz no tratamento de distúrbios alérgicos.

Em uma revisão bibliográfica que cita frutos amazônicos com potencial efeito sobre a COVID-19 por modulação inflamatória. Na pesquisa, os autores citam que o Camu-camu seria uma boa escolha como alimento eficaz que pode auxiliar a tratar as complicações do COVID 19 em pacientes idosos, por conta das suas atividades biológicas devido ao efeito conjunto de seu teor de fitoquímicos como polifenóis e antocianinas, e minerais como potássio que aumentam a biodisponibilidade de vitamina C que o mesmo apresenta (Manica-Cattani et al., 2022).

No livro escrito por Ribeiro e Da Cruz (2010) cita que apesar dos estudos escassos feitos apenas com o Camu-camu, o mesmo apresenta propriedades antioxidantes, anti-inflamatórias, que são cardioprotetoras, preventivas e terapêuticas de câncer como o de próstata e o de mama e ação na proteção de distúrbios gastrointestinais, na desintoxicação corporal.

Fracassetti e colaboradores (2013) realizaram em seu estudo a avaliação o teor de polifenóis e vitamina C e a capacidade antioxidante do pó da polpa desidratada e da farinha seca obtida a partir da casca e dos resíduos de sementes remanescentes após o preparo da polpa do Camu-camu. A pesquisa apresenta que o teor de fenólicos da farinha de Camu-camu foi superior ao da polpa em pó. Em ambos os produtos foram detectados o flavonol miricetina e conjugados, ácido elágico e conjugados e elagitaninos. A cianidina 3- glicosídeo e a quercetina e seus glicosídeos foram encontrados apenas no pó da polpa, enquanto os proantocianinos estavam presentes apenas na farinha (tabela 3). O teor de vitamina C foi menor na polpa em pó do que na farinha.

Tabela 3. Análise HPLC–DAD–ESI-MS–MS de compostos fenólicos de Camu-camu em pó.

Numero	Composto	Tempo de retenção (min)	[M-H]	λmáx (nm)	Fragmentos de MS
Flavonóis	Miricetina 3-O-hexosídeo	27,5	479	264, 358	316, 221, 179
4					
6	Miricetina 3-O-pentosídeo	29,2	449	258, 356	317
6	Miricetina 3-O-pentosídeo	30,4	449	272, 356	316
26	Quercetina 3-O-hexosídeo	31,7	463	256, 362	417, 301
27	Quercetina 3-O- pentosídeo	35,5	433	256, 352	301, 179, 151
28	Miricetina	38,3	317	256, 374	317, 179, 151
Antocianinas	Cianidina 3-O-glicosídeo	20.6	447	520	285
29					
Derivados do ácido elágico					
	Dilactona de ácido valonéico	15,4	469	255, 374	425
1	Hexosídeo de ácido elágico	24,7	463	255, 362	301
2	Pentosídeo de ácido elágico	28,9	433	254, 360	301
3 5	Desoxihexosídeo de ácido elágico	29,7	447	254, 364	300
7	Ácido elágico	30,9	301	256, 368	229
8	Acetil ramnosídeo elágico	35,9	489	254, 362	301
9	Acetil ramnosídeo elágico	36,5	489	254, 362	301
10	Derivado de ácido elágico	37,2	585	254, 360	415, 301
12	Derivado de ácido elágico	40,8	719	254, 362	301
13	Derivado de ácido elágico	41,5	719	254, 362	301
Elagitaninos	Vescalagina	11,5	933	246	915, 889, 631
15					
16	Castalagin	13,9	933	246	915, 889, 631
48	HHDP-galoil-glicose	15.3	633	240, 270	463, 301
18	Di-HHDP-glicose (pedunculagina)	16.2	784	240	481, 301
47	Di-HHDP-galoil-glicose (casuarictina/potentilina)	19,5	935	240, 270	917, 633, 301

19	HHDP-galoil-glicose	19,8	633	240, 270	463, 301
20	Di-HHDP-galoil-glicose (casuarictina/potentilina)	20,5	935	240, 270	917, 633, 301
21	Digaloil-HHDP-glicose	21,8	785	240, 272	484, 301
23	Di-HHDP-galoil-glicose (casuarictina)	25,9	935	240, 270	917, 633, 301
24	Tri-galoil-HHDP-glicose (telimagrandina II)	27,9	937	240, 276	767, 741, 465, 301
Derivados do ácido gálico 14	Ácido gálico	9,0	169	274	125
25	Derivado de ácido gálico	37,8	569	238, 274	551, 523, 169
Proantocianidinas 17	Galocatequina-galocatequina galato-galocatequinaalato 46	15,8	1221	240.274	915
	Galocatequina-galato 22	24,0	457	240, 274	331.305.169
	Dímero de galocatequina-galato	24,1	915	240, 274	457, 169

FONTE: Fracassetti et al., 2013

2.6 *Drosophila melanogaster* como modelo animal para estudos de segurança

Drosophila melanogaster é um dos organismos experimentais mais comumente usados e foi estudado experimentalmente pela primeira vez pelo Dr. Castle (Castle 1906) e usado pelo Dr. Morgan para experimentos genéticos a partir de 1909 (Yamaguchi; Yoshida, 2018).

Nos últimos 25 anos, a mosca da fruta, *Drosophila melanogaster*, emergiu como um dos principais modelos para estudar as interações hospedeiro-micróbio. Graças, em parte, ao alto grau de conservação evolutiva entre as vias de sinalização e sistemas de órgãos de mamíferos e moscas, estudos usando o modelo de *Drosophila* lançaram luz não apenas sobre os mecanismos moleculares básicos de reconhecimento de patógenos e sinalização de um fator nuclear, mas também sobre a natureza das respostas fisiológicas ativadas no hospedeiro pela infecção e como a desregulação dessas respostas contribui para a doença (Troha e Buchon, 2019).

De acordo com o estudo de Miguel-Aliaga e colaboradores (2018) que realizou um artigo de revisão sobre o trato gastro-intestinal da mosca, o “plano básico” do intestino de *Drosophila* adulta consiste em um tubo revestido por uma monocamada epitelial. Consiste em quatro tipos de células: células-tronco intestinais (ISCs), CEs absortivas, células EE secretoras e enteroblastos (Ebs). É importante notar que as células epiteliais do intestino médio têm um arranjo reverso das junções em comparação com outros epitélios de *Drosophila*, com junções oclusivas acima das junções aderentes, como nos vertebrados. As especializações anatômicas e a compartimentalização regional permitem ingestão, armazenamento, digestão, absorção e defecação sequenciais. Como nos mamíferos, as diferentes partes do trato digestivo da *Drosophila* são altamente especializadas em anatomia, organização e função.

A *Drosophila melanogaster* foi estabelecida como um modelo emergente e valioso na pesquisa experimental de alimentos e nutrição nas últimas décadas. A mesma é adequada para ser usado em estudos de intervenção nutricional, pois apresenta muitas semelhanças com espécies de mamíferos. Embora o plano corporal do inseto seja mais simples do que o dos mamíferos, a anatomia da mosca-da-fruta inclui sistemas de órgãos com funções equivalentes do coração, pulmão, rim, fígado e gônadas dos mamíferos. E também possui um intestino complexo e dinâmico semelhante em estrutura e organização ao intestino dos mamíferos. Além dos tecidos, a fisiologia e anatomia dos intestinos de mamíferos e *D. melanogaster* exibem propriedades semelhantes. Inclusive possui um sistema nervoso central e periférico, produz hormônios gastrointestinais e sexuais como peptídeos semelhantes à insulina, hormônio juvenil e ecdisona que afetam o metabolismo e o desenvolvimento da mosca, e ocupa genes e proteínas exibindo 50-60% de ortologia para os mamíferos (Staats et al., 2018).

A mosca *Drosophila melanogaster* é um modelo alternativo muito eficiente para avaliar a toxicidade de substâncias (Musachio et al., 2020). Levando em consideração ao princípio dos 3R's (Tréz, 2018) salientando refinar e anemizar a dor ou o sofrimento animal, tem sido utilizado modelos animais alternativos, como a mosca *Drosophila melanogaster*. A *Drosophila melanogaster* possui um complexo e dinâmico sistema digestório semelhante em estrutura e organização ao dos mamíferos, apesar do plano corporal do inseto ser mais simples do que o dos mamíferos, a anatomia da mosca inclui sistemas de órgãos com funções do coração, pulmão, rim, fígado e gônadas, facilitando a comparação no estudo científico (Staats et al., 2018). Além disso, o modelo apresenta um manejo de criação fácil, ciclo de vida rápido e é isento da aprovação de comitê de ética para a realização de estudos. Complementar a isso, a mosca tem se destacado em estudos toxicológicos através da avaliação de biomarcadores de

estresse oxidativos, além de ser um modelo de referência em termos de futuras direções de pesquisa aplicada a alimentos e nutrição.

3. OBJETIVOS

3.1 Objetivo geral:

Analisar a segurança e o efeito antioxidante do Camu-camu (*Myrciaria dubia*) em *Drosophila melanogaster* expostas ao ferro.

3.2 Objetivos específicos:

- Expor as moscas em mais de uma curva de concentração estabelecida de Camu-camu para avaliar sua segurança e toxicidade;
- Expor também ao Ferro em uma concentração de 20 mM no tempo de 24 horas para analisar os efeitos de prevenção que o pó do Camu-camu pode ocasionar em um organismo vivo exposto ao dano oxidativo;
- Realizar testes comportamentais nas moscas expostas as concentrações de Camu-camu e ao Ferro para analisar possível dano locomotor de ambos,

4. RESULTADOS

Os resultados que fazem parte dessa dissertação de mestrado estão apresentados na forma de manuscrito. As seções descrevendo Materiais e Métodos, Resultados, Discussão e Referências encontram-se no próprio manuscrito.

Evaluation of the antioxidant activity of Camu-camu (*Myrciaria dubia*) powder in *Drosophila melanogaster* under basal redox status and under oxidative stress

Authors: Rafaela Garay Pires^a, Elize Musachio^a, Eliana Jardim Fernandes^a, Stefani Andrade Luana Barreto Meichtry^a, Dieniffer Espinosa Janner^a, Ivana Berenice Mânica da Cruz^b, Graziela Moro Meira^b, Fernanda Barbisan^b, Marina Prigol^{a*}

^a Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas (LaftamBio)- Universidade Federal do Pampa (UNIPAMPA), Campus Itaqui.

^b Laboratório de Biogenômica- Universidade federal de Santa Maria (UFSM)

*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

E-mail addresses: marinaprigol@unipampa.edu.br (M. Prigol)

ABSTRACT

Camu-camu powder is marketed as a food supplement and, like all substances, it is essential to evaluate the dosages regarding its safety. We exposed second-stage larvae to different concentrations of Camu-camu powder and, after 48h, we obtained an (lethal concentration) LC₅₀ value of 4,799 mg/mL. It was observed that the larvae were not able to fully develop at concentrations above 5 mg/mL as evidenced by the reduction in the rate of pupation and hatching, due to toxicity evidenced by alterations in markers of oxidative stress, antioxidant defenses and behavioral analysis. In addition to this, we chose the concentration of 0,5 mg/mL (10X lower than the LC₅₀), as it was shown to be safe in the first moment of this work, and we performed analyzes to demonstrate its protective action against the oxidative effect of Fe. It was found that reactive species production and lipid peroxidation were preserved by Camu-camu at the control level when flies were exposed to Fe, along with SOD, CAT and GST enzyme activity. Camu-camu powder also minimized the possible mortality of flies when exposed to Fe, preserved cell viability and protected flies from developing changes in behavior and AChE activity. The Camu-camu powder made the *Drosophila melanogaster* organism resistant to oxidative insults generated by exposure to Fe, demonstrating therapeutic and preventive treatment potential, suggesting that its consumption during development, in adequate concentrations, can develop greater resistance to stressful photogens.

Keywords: *Drosophila melanogaster*; Camu-camu, oxidative stress; antioxidant

1. INTRODUCTION

A diet rich in fruits and vegetables has been shown to be beneficial for the prevention of numerous non-communicable diseases, as well as for long-term health (Khan et al., 2022; Jaglan et al., 2022) due to therapeutic effect attributed to the content of bioactive phytochemicals (Sosa et al., 2019). In this sense, the Amazon stands out worldwide as the richest biome in the biodiversity of fruit species, which have a series of bioactive molecules in their composition, with promising biological activities (Santos et al., 2022). Among the various Amazonian fruits, we highlight Camu-camu (*Myrciaria dubia*), a fruit of a tree typical of the Amazon, which grows on the banks of rivers, streams, lakes and mangroves (Neri-Numa et al., 2018). The Camu-camu is a source of potassium, iron, calcium and phosphorus and various types of amino acids such as serine, valine and leucine (Zapata & Dufour, 1993). However, it has gained notoriety due to its high concentration of polyphenols, carotenoids and anthocyanins, but mainly because it is the fruit with the highest concentration of Vitamin C (Abot et al., 2022, Akter et al., 2011; Pardo-Aldave., 2019; Genovese et al., 2009).

Currently, Camu-camu has gained the trust of consumers due to its various health benefits. Its beneficial action is attributed to its high concentration of antioxidants (mainly to the high levels of phenolic compounds and acid ascorbic), and thus has drawn the attention of several researchers interested in its benefits (De Abreu Figueiredo et al., 2020; Chang, Alasalvar, Shahidi, 2019). The consumption of *in nature* Camu-camu is limited by its bitter taste, and the commercialization of the fruit is compromised due to the perishability resulting from the high water activity, which makes it more susceptible to chemical and enzymatic degradation, causing nutritional and commercialization losses (Santos et al., 2022; Neves et al., 2017). Pasteurized pulp is also used for the production of powders (dehydrated pulp) due to the ease of transport, mainly for export, and also due to the practicality of its use in the food industry (Fracassetti et al., 2013; Santos et al., 2022).

Camu-camu has a variety of bioactive compounds, in which we believe in the potential for protection and preservation of the organism against an oxidative insult. In this sense, oxidative stress has been identified as an etiological factor for a wide range of medical conditions, and the use of phytochemicals can positively modulate the course and progression of many diseases (Vitorivic et al., 2021). Cells have antioxidant defense mechanisms that maintain redox balance. Under physiological conditions of oxidative stress, there is an imbalance between the production of reactive species and antioxidant defense, and at this point, exogenous antioxidants, such as those derived from food, can help reduce the stress condition.

Antioxidants are molecules that neutralize free radicals, or reinforce the innate antioxidant capacity of cells (Nimse et al., 2015). Many studies showing the effects of hoopoe and Camu-camu fruit, however, even though the powder is widely commercialized, research evaluating its action is still scarce. Therefore, it is important to examine the effects of Camu-camu powder on cellular redox status under basal (normal) conditions (to judge its potential as a preventive treatment), and also its application for the reduction of chronic oxidative stress. This double evaluation will reveal possible beneficial or harmful effects of the powder under different conditions.

There is a gap related to safety doses of Camu-camu, because due to its sensory characteristics it is little directly consumed, but its pulp is applied to many products and various forms of processing are applied to it. The use of powder as a food supplement is already a reality, but even natural products when consumed in excess can cause a toxic effect due to the presence of various bioactive substances. Toxicity is a question of dose, and thus, toxicological risks may be imminent when a substance is consumed above the limit, even vitamins and minerals (Rietjens et al., 2002). For this, it is important to carry out research that shows concentrations possibly toxic to living organisms, of any product, whether natural or not, in order to establish safe doses.

To demonstrate these effects of Camu-camu powder, we used the fly *Drosophila melanogaster*, an alternative vertebrate model, very efficient to assess the toxicity of various substances related to oxidative stress (Musachio et al., 2020). The fly has enzymatic and non-enzymatic antioxidant systems similar to mammals, and therefore has been highlighted in research related to the evaluation of the effect of bioactive molecules against oxidative stress (Poetini et al., 2018). Therefore, this work has two objectives, where we first aim to evaluate the safety issue regarding the concentration of Camu-camu powder for *in vivo* organism in basal redox status, and from that, to evidence its protective action against oxidative stress in *Drosophila melanogaster*.

2. MATERIALS AND METHODS

2.1 *Drosophila melanogaster* **stok**

The study was carried out using *Drosophila melanogaster*, from the Harwich strain. Fifty stage 2 larvae were allocated to each treatment glass, so that they develop according to the exact

amount of camu-camu powder. They were raised and fed in a controlled temperature environment of 25 ± 1 °C and 60-70% humidity in a circadian cycle of 12 hours of light / 12 hours of darkness.

2.2 Experimental protocol with Camu-camu powder

The Camu-camu powder was purchased from the sales website of the company iHerb® of the brand Navitas Organics®. According to the company: “Our camu berries are wildcrafted and grown organically in their native region of Peru. After ripening on the bush, they are gently harvested, low temperature dehydrated, and then milled into a whole-fruit powder. We use low temperatures and minimal processing from start to finish in order to preserve the camu berries' potent nutrients, and all of our nutritional testing is done on the finished product by third party, independent labs. The berries are gently washed, dehydrated, and then milled into a fine, whole-berry powder. Our Camu Powder is certified USDA Organic, Kosher, and non-GMO.”

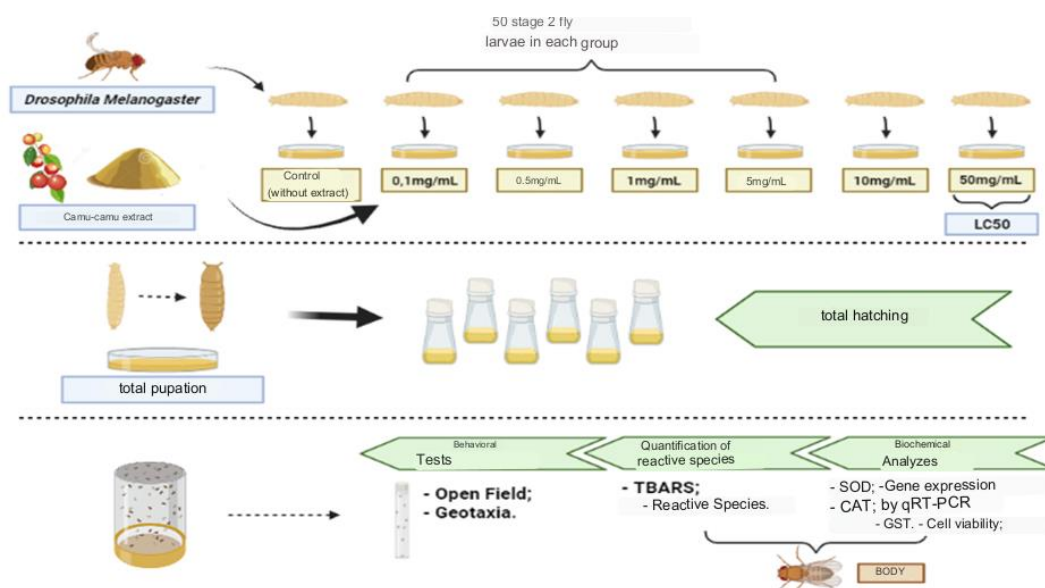


Figure 1. Experimental protocol with Camu-camu powder

Table 1. Nutritional value in 5g of Camu-camu powder

		%DV
Total Fat	0g	0%
Saturated Fat	0g	0%
Trans Fat	0g	
Cholesterol	0mg	0%
Sodium	15mg	1%
Total Carbs	4g	1%
Dietary Fiber	1g	4%
Total Sugar	0g	
Includes Added Sugar	0g	0%
Protein	0g	
Vitamin D	0mcg	0%
Calcium	4mg	0%
Iron	0mg	0%
Potassium	39mg	0%
Vitamin C	682mg	760%

2.3 Lethal concentration (LC₅₀)

The lethal concentration that killed 50% of the exposed animals (LC₅₀) was performed according to the study by Musachio et al. (2021). 2nd stage larvae of *Drosophila melanogaster* were exposed to 6 different concentrations of Camu-camu: 0.1, 0.5, 1, 5, 10 and 50 mg/mL homogenized with your standard diet in a petri dish. Each exposed group contained 50 larvae, 6 independent experiments were performed (n = 6). After 48 hours, the number of dead flies in each experimental group was counted.

2.4 Exposure treatment with Iron

The iron (Fe) exposure treatment was carried out according to the study by Poetini et al. (2018) with adaptations. Thirty *Drosophila melanogaster* flies of both sexes were used and separated into four groups: (1) Control, (2) Camu-camu, (3) Fe, (4) Camu-camu + Fe. The flies were acutely exposed to the treatment in a diet containing Fe in the form of ferrous sulfate on a filter paper, in which a concentration of 20 mM and 1% of sucrose was added, the flies were exposed for 24 hours.

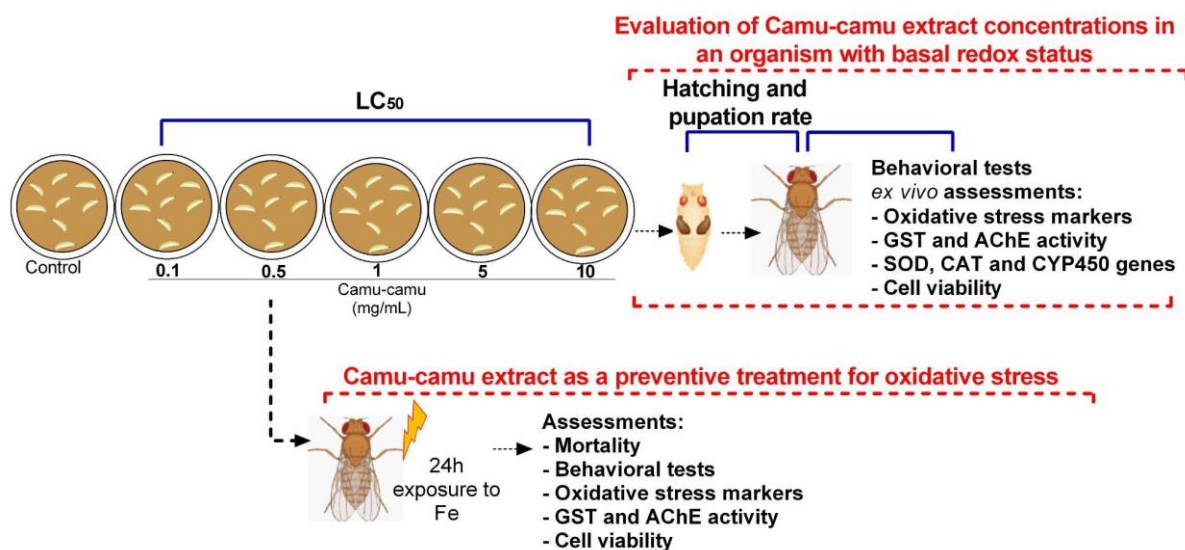


Figure 2. Schematic representation of the experimental design.

2.5 Pupation and hatching rate

Fifty second-stage larvae were added to the respective diets, which remained in the medium until they evolved into the pupal stage. As the larvae were transforming into pupae, they were transferred to flasks containing a standard diet, and remained there throughout the entire metamorphosis until the time of hatching. At that moment, the number of larvae that managed to reach the pupal stage was counted. After that moment, it was counted from the number of pupae formed, the amount that hatched, turning into flies.

2.6 Behavioral tests

2.6.1 Open field test

Open field behavioral tests were performed, as described by Connolly (1966) and adapted by Musachio et al. (2020). Fifteen flies from each group from four independent experiments were used, totaling 60 flies per group used for the test application.

The flies were lightly cryoanesthetized in a falcon tube and separately added to a transparent polycarbonate petri dish (9 mm in diameter) with the aid of a soft bristle brush. The plate cover was graduated with squares measuring 1 cm² each, without spacing, slightly marked with a thin line, so it would not interfere with the behavior of the flies during the test. The test started 2 min after the flies were added to the plate. This amount of time is appropriate to reestablish the flies' anesthesia and acclimate them to the plate, so it would not interfere with

the test. After 2 min, the open field test was performed. Thus, in order to evaluate the spontaneous locomotor activity of the flies, the number of quadrants walked by the flies was observed and visually counted with the help of a manual counter, triggered at each crossed square by the fly, during 60 s timed with the aid of a digital chronometer. The test was performed in duplicate and the mean values were calculated.

2.6.2 Negative geotaxis

Negative geotaxis test was performed according to the study by Paula et al. (2012) with adaptations. The test was performed on five flies from each group, from four independent experiments. The flies were anesthetized and transferred individually to tubes. After 10 min of recovery, the tubes (one at a time) in an upright position were lightly tapped on a flat platform so that the flies remained at the base of the tube.

Then the timer was quickly triggered beginning the count of the time spent by each fly to reach the height of 8 cm, measured from the base of the tube. Each fly had a time maximum of 120 s to scroll the apparatus until reaching 8 cm of height. The time interval between one repetition and another for each fly was 3 min. During the test, the flies remained in their respective tubes. The test was repeated five times per fly, and the average of the times was calculated individually for the statistical analysis.

2.7 Quantification of reactive species

The determination of reactive species levels (RS) was performed according to the study by Pérez-Severiano et al., (2004). Twenty flies were homogenized in 1 mL of Tris buffer (10 mM, pH 7.0) and centrifuged at 1004G for 5 min. An aliquot of 34 μ L sample supernatant was added to a mixture containing 964 μ L HEPES buffer (pH 7.0) and 10 μ L 2,7-dichlorofluorescein diacetate (3,33M; DCFDA). Therefore, after 1 h of incubation, the emission of fluorescence from the DCFH resulting from the oxidation of DCFDA was monitored in a spectrophotometer in an excitation wavelength of 488 nm and an emission wavelength of 520 nm. The results obtained were expressed as a percentage of the control. The average of three independent experiments was used totaling 60 flies for each group.

2.8 Evaluation of lipid peroxidation by the method of quantification of reactive species to thiobarbituric acid

The evaluation of lipid peroxidation by the method of quantification of reactive species to thiobarbituric acid was performed according to the study by Ohkawa et al., (1979) with adaptations. Were homogenized in 500 μ L HEPES buffer and centrifuged at 1000g for 10 min. An aliquot of supernatant (100 μ L) was added to test tubes containing 125 μ L of thiobarbituric acid (TBA, 8%), 12 μ L acetic acid, 50 μ L of sodium dodecyl sulfate (SDS, 1.2%), and 25 μ L distilled water. The mixture was incubated in a water bath at 95 °C for 2 h. The reaction product (200 μ L) was transferred to microplate acrylic and read in a spectrophotometer at 532 nm. The results were expressed nmol MDA/mg of tissue.

2.9 Superoxide dismutase, catalase and glutathione-s-transferase activity

2.9.1 Determination of Superoxide dismutase activity

The activities of antioxidant enzymes such as superoxide dismutase (SOD) of according to the study of Kostyuk and Potapovich (1989) with modifications, by monitoring the inhibitory effect of SOD on quercetin oxidation. To produce the analysis samples, an aliquot of 10 μ L of the supernatant was diluted in 90 μ L of HEPES buffer. The reaction solution comprised sodium phosphate buffer (0.025M/0.1 mM EDTA, pH 10) and N, N, N Tetramethylethylenediamine (TEMED) was added (1 mL) along with 10 μ L of diluted sample and 50 μ L of quercetin in the cuvette for spectrophotometer reading at 406 nm for 2 min. The results were corrected for the absorbance of the amount of protein present in the supernatant sample, calculated as percentage inhibition of the oxidation of quercetin. The final result was expressed in the SOD unit per mg/protein (U/mg protein)

2.9.2 Determination of Catalase activity

The activities of catalase (CAT) were performed according to Aebi (1987), adapted by Paula et al. (2012), which consists of the enzyme's ability to degrade H₂O₂. It was added 30 μ L of supernatant to a quartz cuvette along with 2 mL of reaction mixture composed of 0.25M KPi buffer/2.5 mM EDTA pH 7.0, and Triton H₂O₂. The reading was performed in a spectrophotometer at wavelength of 412 nm for 2 min. The analysis was performed in 5 independent experiments, with samples in duplicate, and the results were corrected according

to the protein concentration. The final result was expressed in the CAT unit per mg/protein (U/mg protein).

2.9.3 Glutathione-S-transferase activity

The determination of detoxifying enzyme Glutathione-S-Transferase (GST) was performed according to Habig et al. (1974), and evaluation of cell viability (Franco et al., 2009). The technique consists in the catalytic action of GST in the conjugation reaction of 1-chloro-2,4- dinitrobenzene (CDNB) with reduced glutathione (GSH) to obtain a thioether (4-dinitrophenyl glutathione). To perform the reading in a spectrophotometer at 340 nm for 2 min, it was added to an acrylic cuvette 30 μ L of supernatant, 1000 μ L of mix (0.25M KPi buffer/2.5 mM EDTA pH 7.0, 100 mM GSH and distilled water) and finally, 20 μ L of the CDNB primer substrate (50 mM). The results were corrected by value of protein and expressed in nanomol of enzyme activity/mg protein (nmol/mg protein).

2.10 Protein quantification

The biochemical analysis that evaluated the enzymatic activities and the TBARS analysis had the values of absorbance corrected by the value of total protein existing in the samples. For this, the Bradford (1976) method was used.

2.11 Gene Expression by qRT-PCR

After hatching, 30 flies from each treatment were macerated and using Trizol Reagent (Ludwig-Brazil) following the manufacturer's protocol, total RNA was extracted. Then the amount and purity were analyzed using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Del., USA). Then, to perform reverse transcription, RNA samples (1 μ g/mL) were added to 0.2 μ L of DNAase (Invitrogen Life Technologies, Carlsbad, CA, USA) at 37 °C for 5 min, followed by heating at 65 °C for 10 min. cDNA was generated with 1 μ L of Iscript cDNA and 4 μ L of Mix Iscript (Bio-Rad Laboratories, Hercules, CA-USA). The reaction consisted of the following steps: heating at 25 °C for 5 minutes, at 42 °C for 30 minutes and at 85 °C for 5 minutes, followed by incubation at 5 °C for 60 minutes. After obtaining the cDNA, qRT-PCR was performed, as previously described by Ferreira et al. (2022). For each sample, qRT-PCR was performed in triplicate using 1 μ M of each primer, 1000 ng/ μ L of cDNA, water RNase-free and 2x QuantiFast SYBR® Green PCR Master Mix (QIAGEN Biotechnology- Hilden-Germany) whose final reaction volume was 20 μ L. The cycle in the

Rotor Gene® thermocillator (Qiagen-Hilden-Germany) to perform the qRT-PCR was: 95°C for 3 minutes, 40 cycles of 95°C for 10 seconds and 60°C for 30 seconds, and a curve of melting from 60°C to 90°C, with 0.5 °C increments, for 5 s. For each sample, qRT-PCR was performed in triplicate. The GPDH gene was used as the housekeeping gene for the reaction. Values less than 1 indicate downregulation of the gene, values greater than 1 indicate up-regulation of gene expression, when compared to the control group. The specific primer pairs used in this study were designed based on sequences available at GeneBank and synthesized by IDT (Integrated DNA Technologies).

Table 2. Gene primers list.

Genes	Primers	
	Sense	Antisense
GPDH	ATGGAGATGATTCGCTTCT	GCTCCTCAATGGTTTTTCCA
SOD	ACCGCACTTCAATCCGTAG	AGTCGGTGATGTTGACCTTG
CAT	ACCAGGGCATCAAGAATCG	AACTTCTTGGCCTGCTCGTA
CYP18a1	TCTTGTTCTTGGCCGTCGG	AGCAATGTGATCTGCAGCCT

2.12 Statistical analysis

Regarding the statistical analysis, the results were analyzed using the GraphPad Prism software version 8 (San Diego, CA, USA), using one-way or two-way (rt-PCR) analysis of variance (ANOVA). Data normality was analyzed using the Shapiro-Wilk test. For the data that showed a Gaussian distribution, it was verified homoscedasticity by the Bartlett test. Data that presented non-normal distribution or heterogeneous variance, the Kruskal-Wallis was used.

For data with normal and homoscedastic distribution, the Bonferroni post hoc will be used. All values will be expressed as mean $\pm$ standard error of the mean (SEM).

3. RESULTS

3.1 Safety evaluation of different doses of Camu-camu

3.1.1 LC50

The LC50 (Figure 2) of the larvae exposed to different concentrations of Camu-camu powder (0.1, 0.5, 1, 5, 10, 50 mg/mL) was stipulated. The LC50 value was obtained after 48 hours of exposure, which resulted in 4.799 mg/mL.

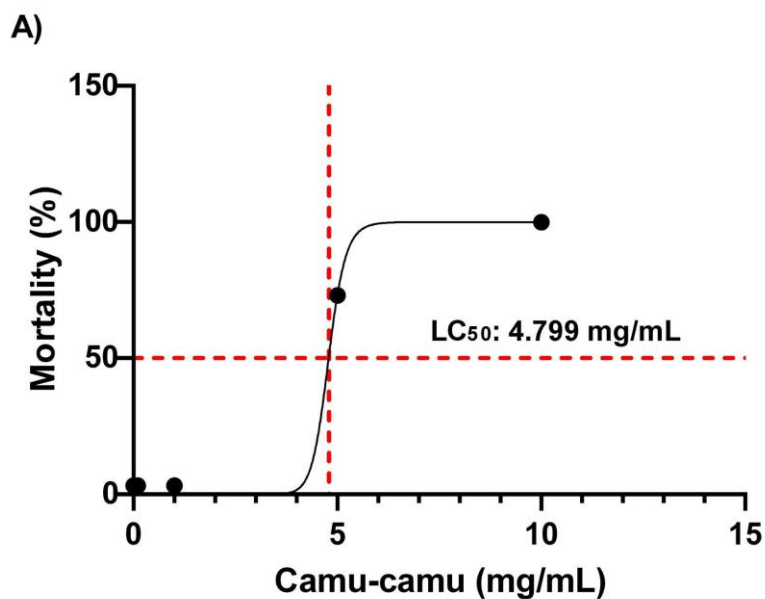


Figure 3. Evaluation of the lethal concentration of Camu-camu that killed 50% (LC50) of exposed larvae. For 48 h, 2nd stage larvae of *Drosophila melanogaster* were exposed to 8 different concentrations of Camu-camu: 0.1, 0.5, 1, 5, 10 and 50 mg/mL.

3.1.2 Hatching and pupation rate

According to the pupation rate (Figure 3 A), there was a reduction in the formation of pupae in the 5 and 10 mg/mL ($P = 0.0008$ and $P = 0.0190$; respectively) groups compared to

the control group. Regarding the hatching rate (Figure 3 B), it also showed a low pupal development for flies also at the highest concentrations of Camu-camu, 5 and 10 mg/mL ($P = 0.0190$ and $P = 0.0008$, respectively).

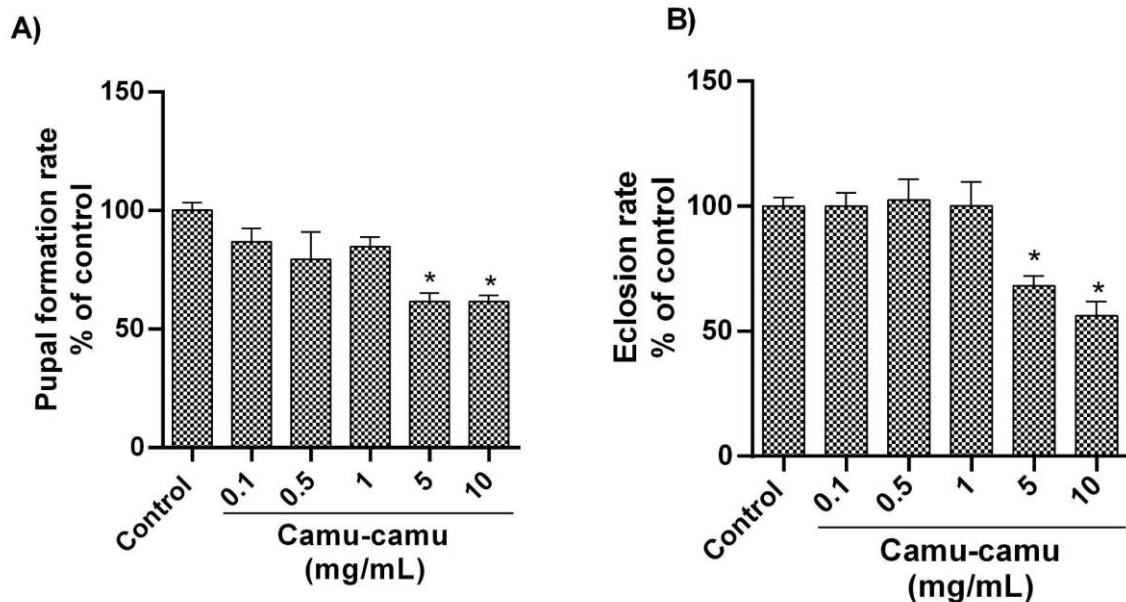


Figure 4. Evaluation of pupation (A) and hatching (B) of *Drosophila melanogaster* exposed to different concentrations of Camu-camu powder. The graphs express the comparison of the different concentrations of Camu-camu in relation to the control group. Statistical difference was considered significant when $P < 0.05$, and indicated in the graphs by the symbol *. Values were expressed as mean $\pm$ standard error of mean (SEM).

3.1.3 Behavioral tests

Regarding the behavioral tests, the open-field (Figure 5 A) test showed a reduction in the highest concentrations of Camu-camu powder, 5 and 10 mg/mL ($P = 0.0346$ and $P = 0.0038$ respectively). However, in the geotaxis test (Figure 4 B), it showed an increase in climbing time also at concentrations of 5 and 10 mg/mL, being different in relation to the control group ($P = 0.0346$ and $P = 0.0038$).

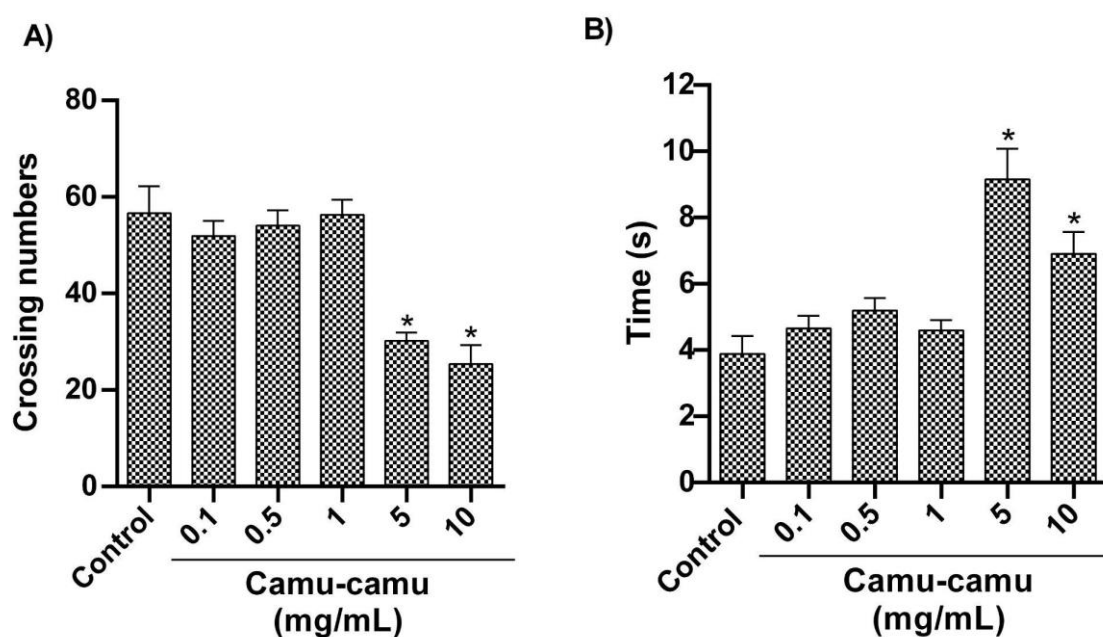


Figure 5. Evaluation of the behavior of *Drosophila melanogaster* exposed during development to different concentrations of Camu-camu. (A) open-field test and (B) negative geotaxis. Graphs express the comparison of different concentrations of Camu-camu in relation to the control group. Statistical difference was considered significant when $P < 0.005$, and indicated in the graphs by the symbol *. Values were expressed as mean $\pm$ standard error of the mean (SEM).

3.1.4 Quantification of reactive species and lipid peroxidation levels

Regarding the quantification of reactive species (Figure 6 A), we can observe that in the group of flies that were exposed to the highest amounts of Camu-camu powder during development, 5 and 10 mg/mL, they showed an increase in levels ($P = 0.0014$ and $P = 0.0016$, respectively) compared to the control group. However, in the analysis of thiobarbituric acid reactive substances (TBARS) that evaluated lipid peroxidation, there was no statistically significant difference in relation to the control (Figure 6 B).

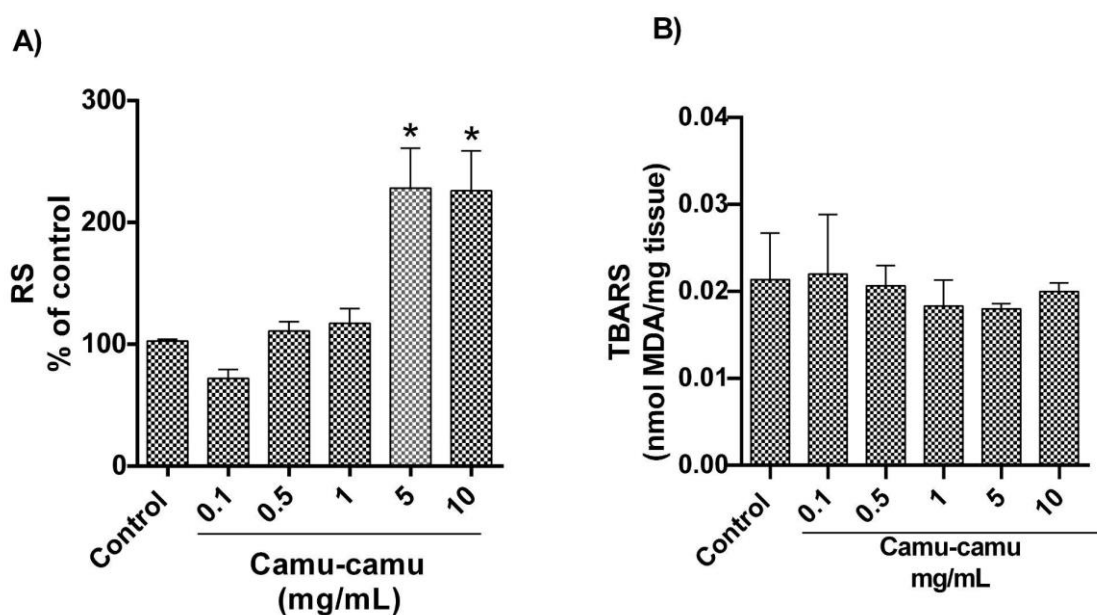


Figure 6. Evaluation of the behavior of *Drosophila melanogaster* exposed during development to different concentrations of Camu-camu. A) Reactive Species and B) thiobarbituric acid reactive species. Graphs express the comparison of different concentrations of Camu-camu in relation to the control group. Statistical difference was considered significant when $P < 0.05$, and indicated in the graphs by the symbol *. Values were expressed as mean $\pm$ standard error of the mean (SEM).

3.1.5 Activity of antioxidant and detoxification enzymes

Regarding antioxidant enzymes, it was observed that flies exposed during development to concentrations of 5 and 10 mg/mL of Camu-camu acquired a reduction in SOD enzyme activity (Figure 7 A) when compared to the control group ($P = 0.0472$ and $P = 0.0272$, respectively). As for CAT (Figure 7 B), an increase in its activity was also observed at concentrations of 5 and 10 mg/mL ($P = 0.0382$ and $P = 0.026$, respectively), when compared to the control group. In the evaluation of the GST detoxification enzyme (Figure 7 C), a reduction in enzymatic activity was observed at concentrations of 5 and 10 mg/mL, when compared to the control group ($P = 0.050$ and $P = 0.0105$, respectively).

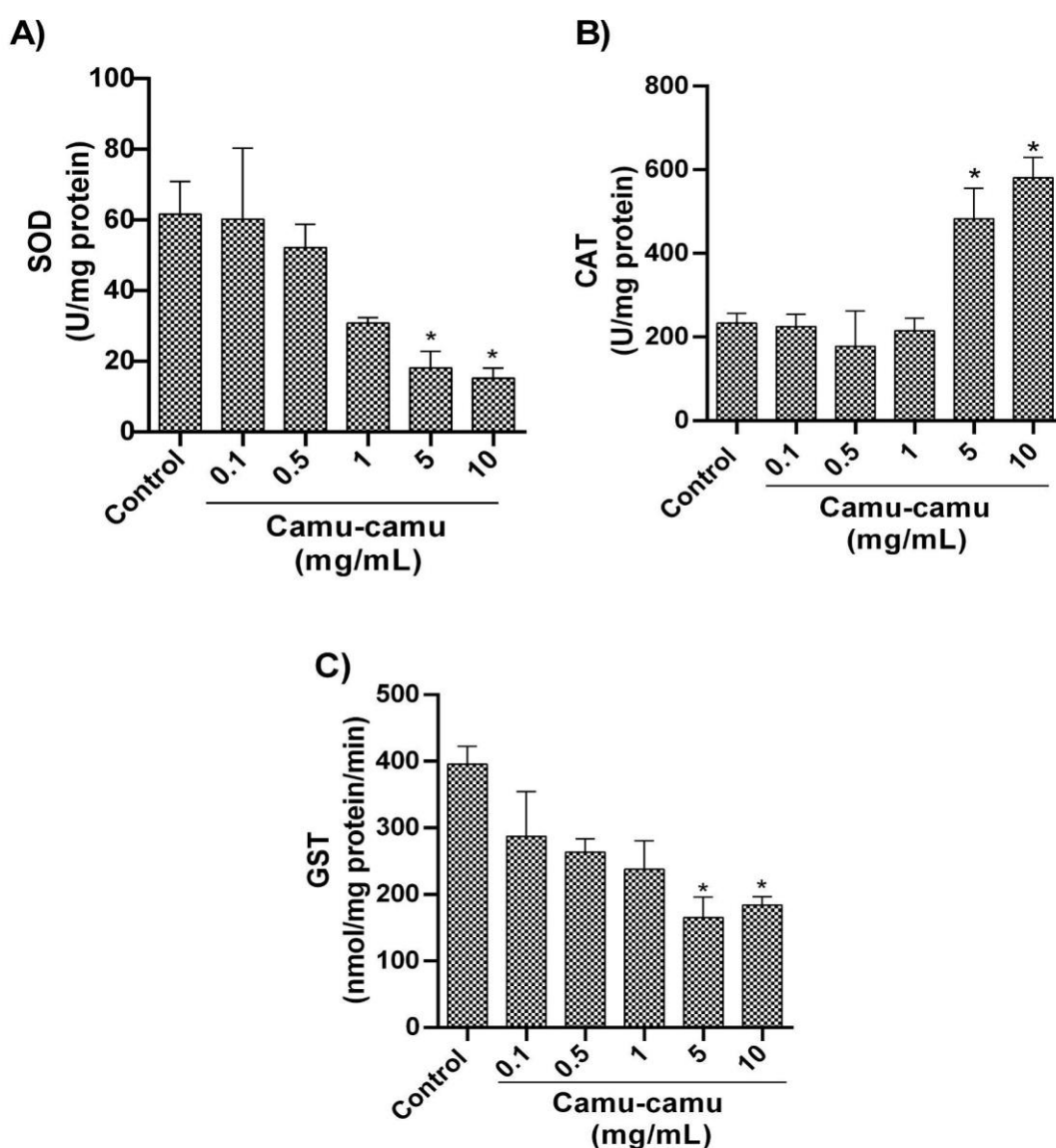


Figure 7. Evaluation of the activity of the enzymes (A) superoxide dismutase (SOD), (B) catalase (CAT) and (C) Glutathione-S-transferase (GST) in *Drosophila melanogaster* exposed to different concentrations of Camu-camu powder. The graphs express the comparison of the different concentrations of Camu-camu in relation to the control group. Statistical difference was considered significant when $P < 0.05$, and indicated in the graphs by the symbol *. Values were expressed as mean $\pm$ standard error of mean (SEM).

3.1.6 Expression of SOD, CAT and Cytochrome P-450 (CYP450) genes

By qRT-PCR analysis, we evaluated the expression of detoxification antioxidant enzyme genes. It was observed that there was an increase in SOD enzyme expression (Figure 8

A) in flies exposed to a concentration of 5 mg/mL, compared to the control group ($P < 0.0001$). However, in CAT gene expression (Figure 8 B), there was no statistically significant difference between groups. As for the CYP450 genes (Figure 8 C), it was observed that there was an increase in expression in flies exposed to all Camu-camu concentrations, 0.1, 0.5, 1, 5 and 10 mg/mL, when compared to the control group ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, $P < 0.0001$, and $P < 0.0001$, respectively).

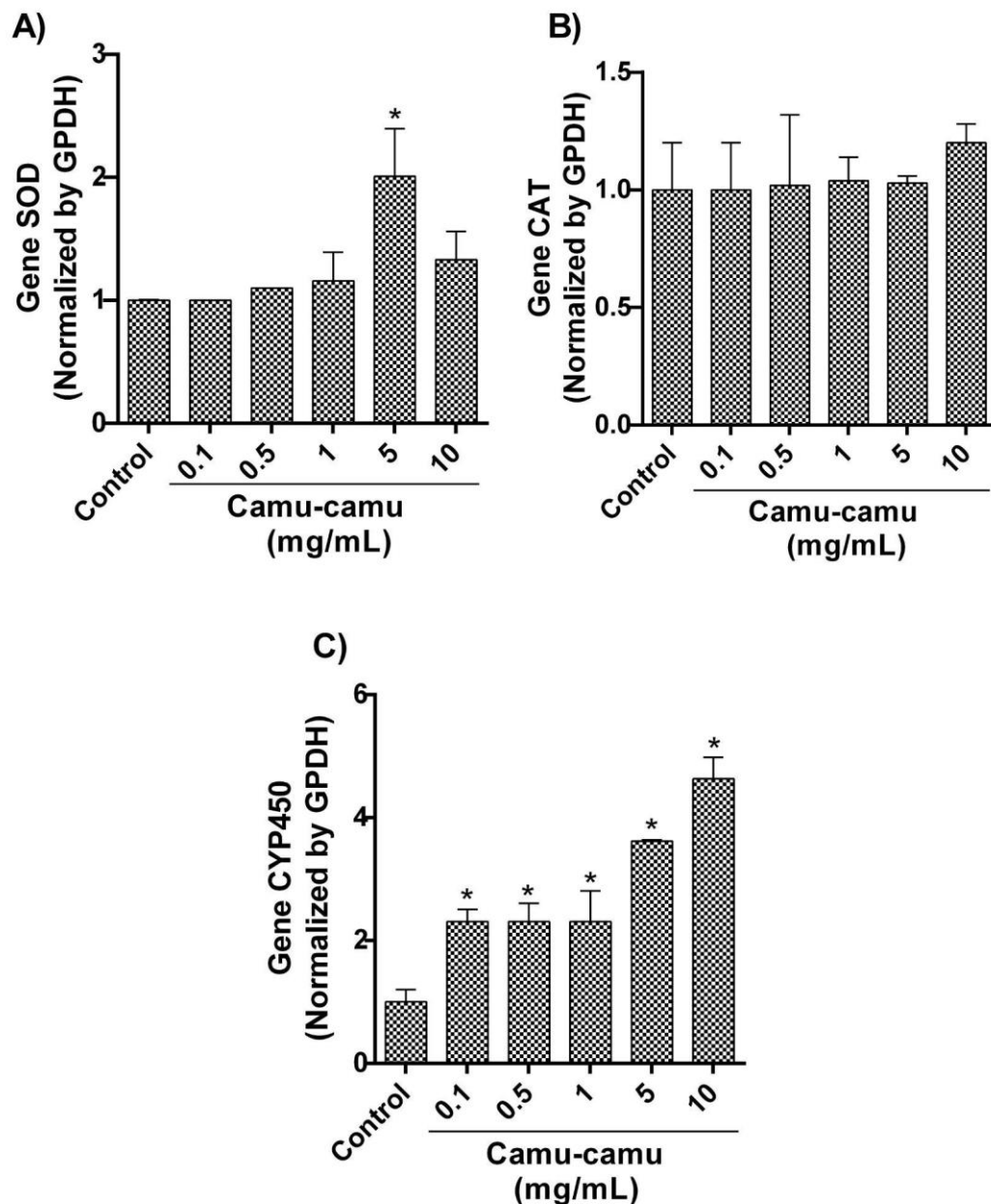


Figure 8. Evaluation of gene expression by analysis and rt-PCR of the enzymes (A) Superoxide dismutase (SOD), (B) Catalase (CAT) and (C) Cytochrome P-450 (CYP450), in flies exposed during development to different concentrations Camu-camu powder. The graphs express the

comparison of the different concentrations of Camu-camu in relation to the control group. Statistical difference was considered significant when $P < 0.05$, and indicated in the graphs by the symbol *. Values were expressed as mean $\pm$ standard error of mean (SEM).

3.1.7 AChE activity

Regarding AChE activity (Figure 9), the high concentrations of Camu-camu powder, 5 and 10 mg/mL, also showed a significant reduction in enzyme activity compared to the control group ($P = 0.0011$ and $P = 0.0014$, respectively).

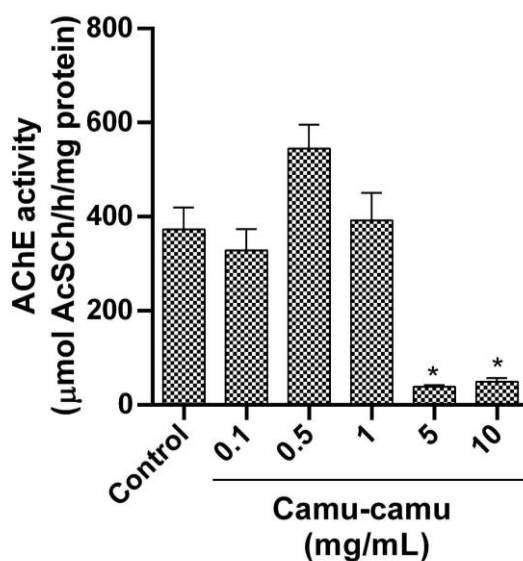


Figure 9. Evaluation of Acetylcholinesterase (AChE) enzyme activity in *Drosophila melanogaster* exposed to different concentrations of Camu-camu powder. The graphs express the comparison of the different concentrations of Camu-camu in relation to the control group. Statistical difference was considered significant when $P < 0.05$, and indicated in the graphs by the symbol *. Values were expressed as mean $\pm$ standard error of mean (SEM).

3.1.7 Cell viability

Cell viability observed by the resazurin test (Figure 10) showed that flies exposed during development to concentrations of 5 and 10 mg/mL had a reduction in cellular metabolic capacity when compared to the control group ($P = 0.0363$ and $P = 0.0220$, respectively).

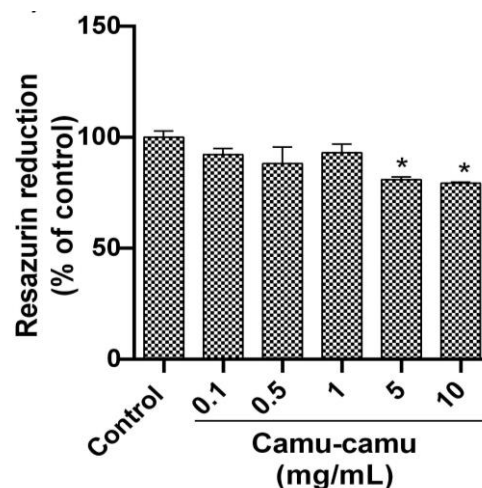


Figure 10. Evaluation of cell viability by the resazurin reduction method in *Drosophila melanogaster* exposed to different concentrations of Camu-camu powder. The graphs express the comparison of the different concentrations of Camu-camu in relation to the control group. Statistical difference was considered significant when $P < 0.05$, and indicated in the graphs by the symbol *. Values were expressed as mean $\pm$ standard error of mean (SEM).

3.2 Protective effect of exposure to Camu-camu powder during development versus exposure to iron after hatching

In order to evaluate the antioxidant protective action of Camu-camu powder in flies exposed during development, a concentration of 0.5 mg/mL was chosen because it did not demonstrate any negative effect on the organism in a basal redox state, and this being a concentration of approximately 10X lower than the concentration obtained in the LC₅₀. Therefore, the group denominated CC in the graphs corresponds to the concentration of Camu-camu powder 0.5mg/mL (flies exposed during the larval development-period). The Fe group corresponds to flies that were not exposed during development to Camu-camu, only to Fe (iron) for 24 hours after hatching. The CC+Fe group corresponds to flies that were exposed to Camu-camu powder 0.5 mg/mL during development, and when hatching, for 24 hours they were exposed to Fe.

3.2.1 Mortality against Fe exposure of adult flies exposed to Camu-camu during development

Mortality of flies exposed during development to a concentration of 0.5 mg/mL, compared to exposure to Fe after hatching. Mortality was evaluated based on 24 h exposure to Fe. Flies exposed only to Fe (with no previous exposure to (Camu-camu)) had a significant increase in mortality compared to the control group ($P < 0.05$). However, flies exposed during development to Camu-camu, and that after hatching, they were exposed to Fe during the 24h (Fe+Camu-camu group), had a lower mortality rate, when compared to the Fe group ($P < 0.0252$).

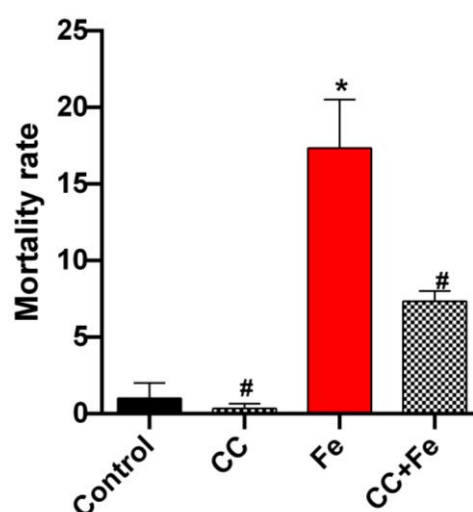


Figure 11. Evaluation of mortality, in 24h of exposure to Iron (Fe), of flies that consumed Camu-camu during development. Data were analyzed using the repeated measures test over time, by two-way ANOVA, followed by Bonferroni's post hoc test. Results were considered significant when $P < 0.05$. Thus, * corresponds to the difference in relation to the group and # shows a difference when compared to the Fe group.

3.2.2 Behavioral tests: locomotor and climbing ability

Regarding the behavioral tests, the open-field test (Figure 12 A) the flies exposed only to the Fe group, obtained a reduction in locomotion compared to the control ($P = 0.0202$). However, the flies that belonged to the CC (0.5 mg/mL) and CC+ Fe group show an increase in locomotor capacity when compared to the group of flies that were exposed only to Fe (no exposure to Camu-camu during development) ($P=0,0047$ and $P = 0,0441$, respectively).

In the geotaxis test (Figure 12 B), it was possible to observe that the flies exposed to Fe had a longer time to climb the tube when compared to the control group ($P = 0.0008$). In the CC+Fe group, no change in climbing ability was observed, as the flies had a shorter time to climb 8 cm, when compared to the Fe group ($P = 0.0016$).

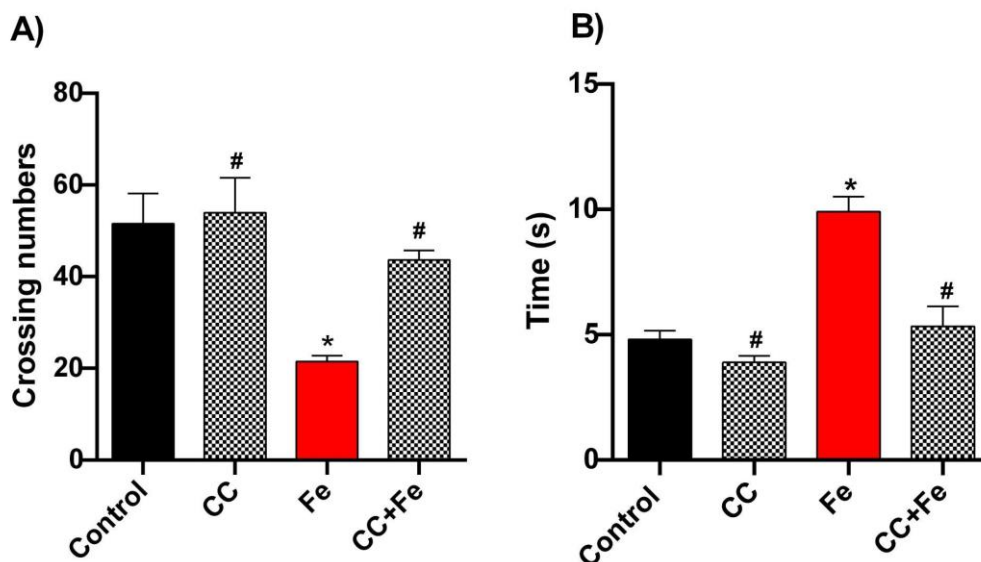


Figure 12. Evaluation of behavioral tests in flies exposed during development to Camu-camu against exposure to Iron (Fe) as adults. (A) open-field test and (B) negative geotaxis. The statistical difference was considered significant when $P < 0.05$, being indicated in the graphs by the symbol * when different from the control group and # when different from the Iron group. Values were expressed as mean $\pm$ standard error of mean (SEM).

3.2.3 Quantification of RS and TBARS levels

It was observed that RS levels (Figure 13 A) are high in flies belonging to the Fe group, when compared to the control ($P < 0.0001$). However, the flies belonging to the CC and CC+Fe groups were similar to the control level, that is, they produced a smaller amount of RS, when compared to the Fe group ($P < 0.0001$ and $P < 0.0001$, respectively).

Regarding lipid peroxidation (Figure 13 B), we can observe that the group exposed only to iron obtained an increase in TBARS levels, when compared to the control group ($P = 0.0077$). However, the flies belonging to the CC and CC+ Fe group, obtained TBARS levels preserved

at the control level, which configured a lower production of TBARS When compared to the Fe group ($P = 0.0032$ and $P = 0.0090$, respectively).

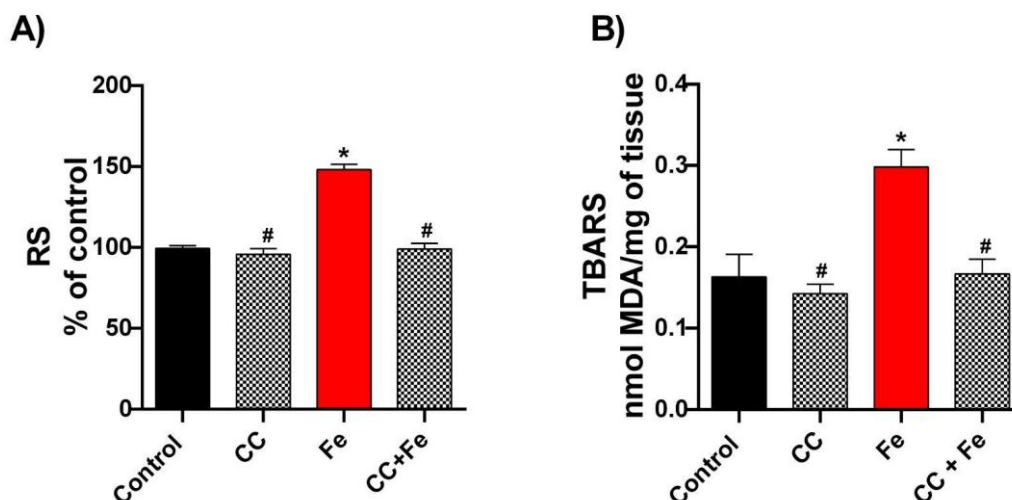


Figure 13. Quantification of (A) reactive species (RS) and (B) lipid peroxidation by quantification of thiobarbituric acid reactive species (TBARS) in flies exposed during development to Camu-camu versus exposure to Iron (Fe) as adults. The statistical difference was considered significant when $P < 0.05$, being indicated in the graphs by the symbol * when different from the control group and # when different from the Fe group (flies not previously exposed to Camu-Camu). Values were expressed as mean $\pm$ standard error of mean (SEM).

3.2.4 Antioxidant enzymes in relation to Fe exposition

According to the results of the antioxidant and detoxifying enzymes, in the evaluation of the SOD enzyme (Figure 14 A) there was an increase in the activity of the enzyme in flies exposed only to Fe, compared to the control group ($P < 0.0001$). In the CC and CC+Fe groups, the flies showed SOD activity maintained the activity at the control level, being lower when compared to the Fe group ($P < 0.0001$ and $P < 0.0001$, respectively).

In the analysis that verified CAT activity (Figure 14 B), flies exposed to Fe showed a reduction in enzyme activity when compared to the control group ($P = 0.0129$).

As for the GST enzyme (Figure 14 C), it was observed that flies belonging to Fe had a reduction in the activity of this enzyme compared to the control group ($P = 0.0032$). However, the CC and CC+Fe groups showed similar GST enzyme activity to the control, that is, there was an increase in activity compared to the Fe group ($P = 0.0068$ and $P = 0.0106$, respectively).

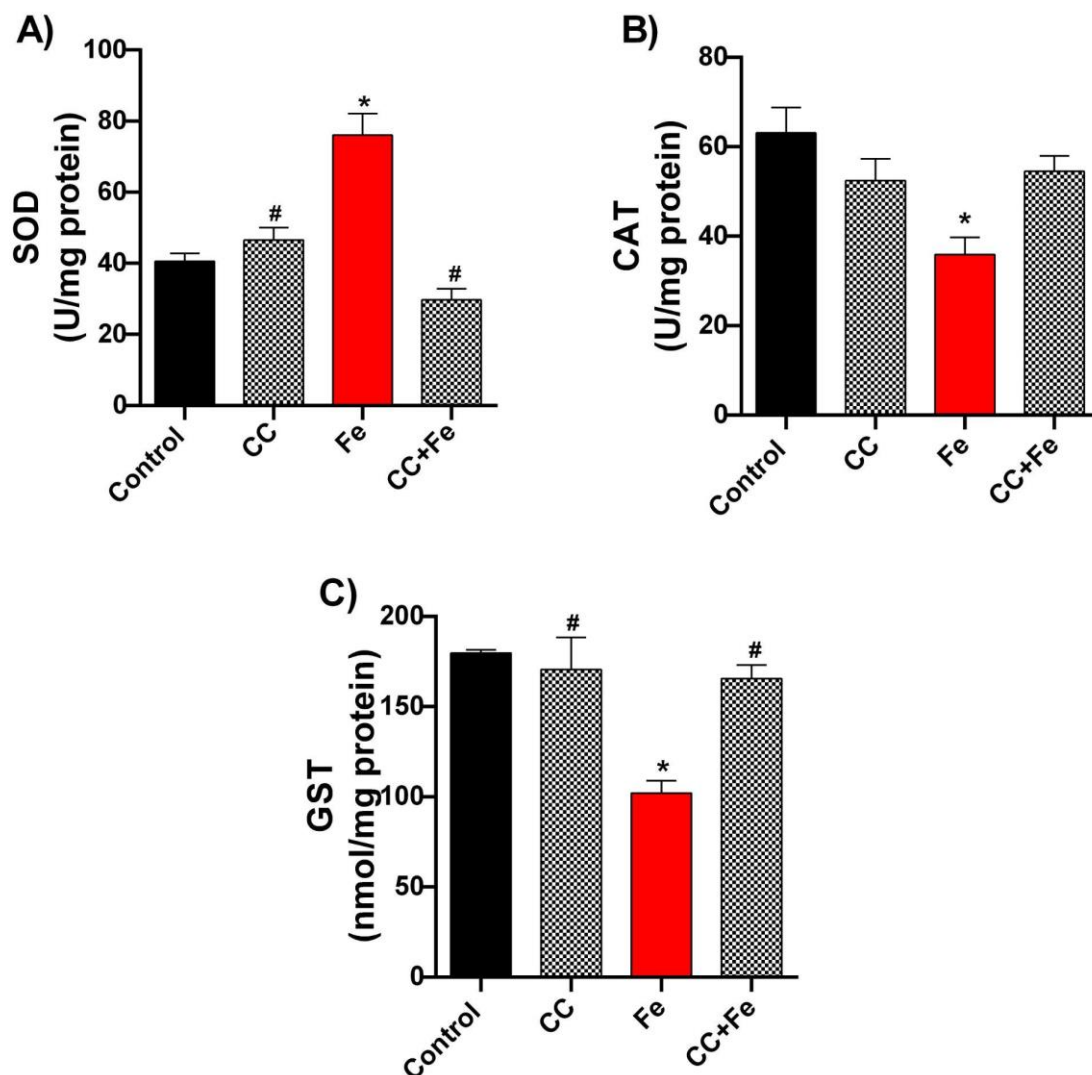


Figure 44. Evaluation of the activity of (A) superoxide dismutase (SOD), (B) catalase (CAT) and (C) glutathione-S-transferase (GST) enzymes in flies exposed during development to Camu-camu (CC) versus exposure to Iron (Fe) as adults. The statistical difference was considered significant when $P < 0.05$, being indicated in the graphs by the symbol * when different from the control group and # when different from the Fe group. Values were expressed as mean $\pm$ standard error of mean (SEM).

3.2.5 AChE activity in relation to Fe exposure

When evaluating the AChE activity (Figure 15), it was observed that the flies exposed only to the Fe group showed a reduction in the activity of the enzyme, when compared to the

control group ($P = 0.0125$). Flies belonging to the CC and CC+Fe groups maintained AChE activity at the control level, however it was increased in relation to the Fe group ($P = 0.0166$ and $P = 0.0007$, respectively).

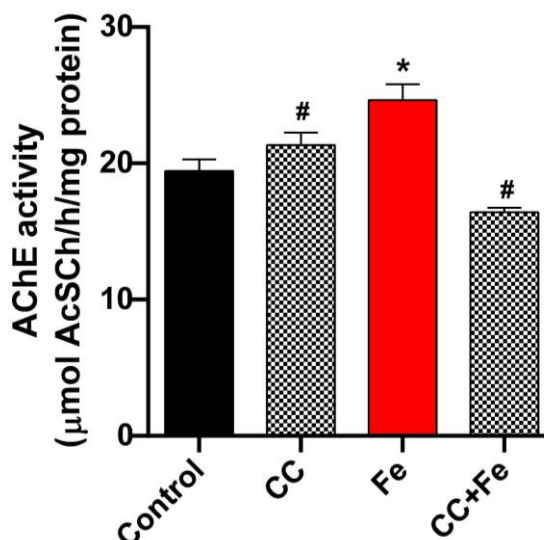


Figure 15. Evaluation of the enzyme Acetylcholinesterase (AChE) activity in flies exposed during development to Camu-camu versus exposure to Iron (Fe) as adults. The statistical difference was considered significant when $P < 0.05$, being indicated in the graphs by the symbol * when different from the control group and # when different from the Fe group. Values were expressed as mean $\pm$ standard error of mean (SEM).

3.2.6 Cell viability in relation to Fe exposure

According to the cell viability test evaluated by the resazurin reduction assay (Figure 16), the flies belonging to the Fe group had a low cellular capacity to reduce resazurin when compared to the control group ($P < 0.0001$). However, the CC and CC+Fe groups showed a greater ability to reduce resazurin, indicating greater cell viability when compared to the Fe group ($P < 0.0001$ and $P > 0.0001$, respectively).

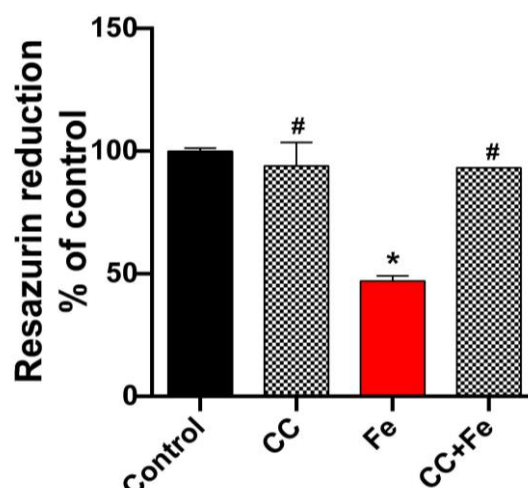


Figure 16. Evaluation of cell viability by the resazurin reduction method in flies exposed during development to Camu-camu versus exposure to Iron (Fe) as adults. The statistical difference was considered significant when $P < 0.05$, being indicated in the graphs by the symbol * when different from the control group and # when different from the Fe group. Values were expressed as mean $\pm$ standard error of mean (SEM).

4. DISCUSSION

Currently, the population has a very hectic routine and with that priority is given to the practicality of having meals with more nutrients and quick preparation, so from that we analyzed the Camu-camu fruit powder, currently marketed as a food supplement. In this sense, we highlight the importance of carrying out studies that evaluate substances that are being consumed without any *in vivo* toxicological reference. Thus, initially we performed an LC₅₀ curve to observe a concentration which can be called safe. Subsequently, we used a concentration 10X lower than the LC₅₀, and we used it to evaluate its protective effect against a model of oxidative stress, induced by exposure to Iron. It is worth mentioning that these estimates were performed in a living organism, the alternative vertebrate model *Drosophila melanogaster*.

Literature searches were carried out on safe concentrations of Camu-camu powder related to reference values and toxicological data, however there is a scarcity of this information. Most existing studies cite the composition of the fruit and its benefits in relation to chronic diseases or that are related to oxidative stress (Do Carmo et al., 2020; Do et al., 2022; Abot et al., 2022), not refer to the fruit powder, which is already being widely used by the population. So, here first an LC₅₀ curve was performed to assess what would be its safe

concentration for the living organism. We initially used second-stage larvae to carry out the initial exposure, as they are models sensitive to oxidative insults, and any alteration implies their development, making the model attractive for our study.

When adding the 2nd stage larvae to different concentrations of Camu-camu, the concentration of 4.799 mg/mL killed 50% of the larvae exposed there during 48h. Based on the LC₅₀, 5 different concentrations of Camu-camu powder were chosen, and parameters of development and oxidative stress were evaluated to first complement the understanding of the action of this product in the organism in a basal redox state. Thus, we tested concentrations of 0.1, 0.5, 1, 5, 10, and 50 mg/mL of Camu-camu powder to first assess safe concentrations.

At these concentrations, we observed the development of the model organism, and found that at higher concentrations there was a reduction in the rate of pupation and hatching. This result means that when exposed to these concentrations, the larvae in the second stage did not complete the development cycle and did not reach the pupal period (observed by the reduction in the formation of pupae), and those that reached the pupal stage could not pass through the metamorphosis, did not form and therefore did not hatch (hence the reduction in hatching). The larva has the ability to repair damage to the imaginal discs induced in the first two stages, however, this possibility of cell regeneration is lost near the end of the third stage (Jaszczak and Halme, 2016). In one study, housefly larvae failed to develop in high concentrations of ascorbic acid due to its pro-oxidant effect (Dhage and Sharbidre, 2021). According to Sotibrán et al. (2011), some flavonoids, in contrast to the beneficial effects, may have harmful effects attributed to the pro-oxidant activity, through the generation of free radicals, under certain conditions. Thus, this deleterious action on the development of the larvae may be related to a pro-oxidant effect induced by the ascorbic acid present in Camu-camu, at these concentrations.

Complementing this, after hatching we observed biomarkers of oxidative stress in adult flies in which they developed at these different concentrations of Camu-camu. Flies exposed during the larval period to two highest concentrations produced a greater amount of RS, however there was no change in the analysis that evaluated lipid peroxidation. In line with these results, the SOD enzyme activity was reduced at these concentrations, while an increase in CAT activity was observed. The term reactive oxygen species is quite generic, and refers to species derived from O₂, which are more reactive than diatomic oxygen, which includes superoxide anion, hydroxyl radical but also non-radical species such as hydrogen peroxide (Jordan et al., 2021). The reduction in SOD activity may indicate that there was no production of superoxide

anion, just as there may have been an increase in the production of H₂O₂, observed by the increase in the activity of the CAT enzyme. On the other hand, the reduction in GST activity at concentrations of 5 and 10 mg/mL may also be related to the presence of high concentrations of ascorbic acid in Camu-camu powder. The protective effects of ascorbic acid are not related to the restoration of GSH levels in cells induced by oxidative stress (Vitorovic et al., 2021), and thus the reduction in GST activity can be attributed to the reduction in GSH levels caused by RS increase.

In agreement with these results, an increase in the expression of the SOD genes was observed only in the concentrations of 5 mg/mL, however, the concentration of 10 mg/mL did not show statistically significant, but it is possible to observe a trend towards an increase in expression also in this group. There was no change in the expression of CAT genes, showing that the increase in enzyme activity is directly related to the production of hydrogen peroxide. Furthermore, we observed that CYP450 gene expression was increased in all groups exposed to Camu-camu during development. This is interesting data, considering that in studies in which animals are exposed during development to toxic products, there is a reduction in CYP450 expression (Giraud et al., 2010; Liao et al., 2023). This process may be an adaptive response of the organism exposed during development to Camu-camu powder, which as an adult may have increased capacity to metabolize and eliminate xenobiotics, due to the possible greater activity of these enzymes. This overexpression can increase the production of reactive species and negatively affect drug efficacy and safety, as it can lead to decreased plasma levels and reduced systemic drug exposure. In general, this increase, as it was greater at concentrations of 5 and 10 mg/mL, should correspond to an increase in RS production, which culminated in a reduction in the metabolism of dehydrogenases to reduce resazurin, also observed in flies exposed to these concentrations during development.

Along with oxidative stress markers, behavioral tests that observe locomotor activity are also well accepted in toxicological studies with *Drosophila melanogaster*. Here, it was evidenced in the open field and negative geotaxis test that the flies exposed during development to concentrations of 5 and 10 mg/mL of Camu-camu powder had reduced locomotion and climbing capacity. To complement the behavioral tests, we evaluated the AChE enzyme of these flies, as it is related to the locomotor capacity exercised by vertebrate animals. AChE enzyme activity was also reduced in groups 5 and 10 mg/mL. It is noteworthy that AChE is very sensitive to oxidative damage, and therefore the reduction may correspond to the pro-oxidant effect exerted by Camu-camu in these two higher concentrations.

These results make it clear that the Camu-camu powder was quite toxic at high concentrations, above 5 mg/mL, at concentrations below 1 mg/mL it did not develop any harm to the fly organism. In the second stage of the research, based on the LC₅₀ and on the analyzes described here, we chose the concentration of 0.5 mg/mL to investigate the protective action of Camu-camu powder against an organism in oxidative stress. Thus, we used an oxidative stress induction protocol, previously described by Poetine et al. (2018), through exposure to Fe. The flies were exposed to Fe in the form of ferrous sulfate on filter paper, to which a concentration of 20 mM and 1% sucrose was added, and they remained there for 24 hours for further analysis. Thus, the Control group was composed of flies that were not exposed during development to Camu-camu or to Fe after hatching, the CC group (Camu-camu) was composed of larvae exposed only during development to a concentration of 0.5mg/ mL of powder, not exposed to Fe after hatching. The Fe group are flies without previous exposure (during development) to camu-camu and the CC+Fe group was composed of flies exposed during development to Camu-camu and when hatching they were exposed for 24 hours to Fe.

We again evaluated the oxidative stress biomarkers, and through these analyzes we observed that the flies belonging to the CC+Fe group had preserved RS and lipid peroxidation levels, in the same way that there was no change in the activity of SOD, CAT and GST enzymes. These findings correspond to the cell viability analysis, where it was possible to observe that there was no change in the metabolism capacity of dehydrogenases to reduce resazurin to resorufin. The protection of the AChE enzyme activity was also unchanged, possibly due to the protection exerted by the Camu-camu powder in the oxidative stress induced by Fe. Locomotor activity, which is affected by oxidative stress as a result of insulting the AChE enzyme, was preserved by prior exposure to Camu-camu powder.

Flies exposed to Fe had a high mortality rate within 24 hours of exposure. However, flies belonging to the CC+Fe group had a lower mortality rate compared to Fe. Our findings are in line with the results obtained in research on antioxidants already well described in the literature, such as the powder of *Ilex paraguariensis*. In this study, the authors also used *Drosophila melanogaster* and exposed it to methylmalonic acid (MMA), a compound in which it is also a model for oxidative stress analysis, and the *Ilex paraguariensis* extract was able to maintain mortality rates at the control level. Another study using the same model of oxidative stress (Fe) induction observed that hesperidin, a flavonoid with high antioxidant potential, was also able to preserve the mortality rate similar to the control. In both studies, the justification for this preservation of the mortality rate is related to the reduction of oxidative stress observed

in these models, as excess reactive oxygen species can be harmful, and cause fatal damage to the living organism (Portela et al., 2019).

In addition, it is worth mentioning that in all analyzes it was possible to observe a perceptive effect exerted by the Camu-camu powder, since in the group that alone an effective redox regulation was observed. Thus, it is important to emphasize that a model organism of *Drosophila melanogaster* was used during the larval period, because it is a period of development, and it is during this period that proteins and the antioxidant system are being formed. However, it was observed that the effect of Camu-camu powder, and that it was effective during development because when adults it was possible to observe a greater resistance to oxidative stress induced by exposure to iron.

5. CONCLUSION

The consumption of its fruit powder as a food supplement is current, and like all substances, it is essential to evaluate doses related to its safety. First, we verified that there was an LC₅₀ of the Camu-camu powder, being 4.799 mg/mL for fly larvae. The two highest concentrations used, 5 and 10 mg/mL, had a pro-oxidant effect, altering the parameters of oxidative stress, which caused locomotor and AChE enzyme damage. It is worth mentioning that both oxidative stress and behavior are related to the survival of individuals, which corresponds to the failure of their larval development cycle, where they died before or during the period of metamorphosis. So far, it has been observed that at these concentrations 5 and 10 mg/mL (above the LC₅₀), there was damage in an organism in redox status under basal (normal) conditions.

Adult flies showed an organism resistant to oxidative insults, and this was attributed to the antioxidant action of the Camu-camu powder, due to the numerous bioactive substances still preserved in the powder. In this way, being effective in oxidative damage, the Camu-camu powder also minimized the possible mortality of flies when exposed to Fe, preserved them from developing changes in behavior and in the AChE enzyme.

Overall, our findings demonstrate the therapeutic and preventive treatment potential exerted by Camu-camu powder in *Drosophila melanogaster*, thus suggesting that its consumption during development, in adequate concentrations, can develop greater resistance to stressful factors.

6. REFERENCES

- ABOLAJI, A. O. et al. Resveratrol prolongs lifespan and improves 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced oxidative damage and behavioural deficits in *Drosophila melanogaster*. **Biochemical and Biophysical Research Communications**, v. 503, n. 2, p. 1042–1048, 2018.
- AEBI, H., 1987. catalase in vitro. **métodos enzimol.** v. 105, p. 121-126.
- BRADFORD, M. M. a rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **anal. biochem.** n. 72, p. 248–254, 1976.
- CASTRO, j. c. et al. myrciaria dubia “camu camu” fruit: health-promoting phytochemicals and functional genomic characteristics. breeding and health benefits of fruit and nut crops, 2018.
- CHANG, h. y. et al. a superexpressão do transportador de monoamina vesicular de drosophila aumenta a atividade motora e a corte, mas diminui o comportamento resposta à cocaína. **mol. psiquiatria.** v. 11, p. 99-113, 2006.
- CHANG, s. k.; alasalvar, c.; shahidi, f. superfruits: phytochemicals, antioxidant efficacies, and health effects—a comprehensive review. **critical reviews in food science and nutrition**, v. 59, n. 10, p. 1580–1604, 2019.
- DE ABREU FIGUEIREDO, j. et al. encapsulation of camu-camu extracts using prebiotic biopolymers: controlled release of bioactive compounds and effect on their physicochemical and thermal properties. **food research international**, v. 137, n. april, p. 109563, 2020.
- DHAGE, P. A.; SHARBIDRE, A. A. Bimodal behavior of ascorbic acid in musca domestica Larvae. **Biointerface Research in Applied Chemistry**, v. 12, n. 4, p. 5199–5216, 2022.
- ABOT, A. et al. Camu-Camu Reduces Obesity and Improves Diabetic Profiles of Obese and Diabetic Mice: A Dose-Ranging Study. **Metabolites**, v. 12, n. 4, 2022.
- DO CARMO, M. A. V. et al. camu-camu (myrciaria dubia) seeds as a novel source of bioactive compounds with promising antimalarial and antischistosomal properties. **food research international**, v. 136, n. february, p. 109334, 2020.
- GENOVESE, M. I. et al. Bioactive compounds and antioxidant capacity of exotic fruits and commercial frozen pulps from Brazil. **Food Science and Technology International**, v. 14, n. 3, p. 207–214, 2008.
- HABIG, W. H.; PABST, M. J.; JAKOBY, W. B. The first. **the journal of biological chemistry**, v. 249, n. 22, p. 7130–7139, 1974.
- JAHROMI, S. R. et al. Neuroprotective effect of decalepis hamiltonii in paraquat-induced neurotoxicity in drosophila melanogaster: Biochemical and behavioral evidences. **Neurochemical Research**, v. 38, n. 12, p. 2616–2624, 2013.
- JASZCZAK, J. S., WOLPE, J. B., BHANDARI, R., JASZCZAK, R. G., & HALME, A. Growth coordination during *Drosophila melanogaster* imaginal disc regeneration is mediated by

signaling through the relaxin receptor Lgr3 in the prothoracic gland. **Genetics**, v. 204, n. 2, p. 703-709, 2016.

JORDAN, A. C., PERRY, C. G., & CHENG, A. J.. Promoting a pro-oxidant state in skeletal muscle: Potential dietary, environmental, and exercise interventions for enhancing endurance-training adaptations. **Free Radical Biology and Medicine**, v. 176, p. 189-202, 2021.

KOSTYUK, V. A.; POTAPOVICH, A. I. Superoxide - driven oxidation of quercetin and a simple sensitive assay for determination of superoxide dismutase. **biochemistry internacional**, v. 19, n. 5, p. 1117–1124, 1989.

LE BOURG, É.; FOURNIER, D. Is lifespan extension accompanied by improved antioxidant defences? A study of superoxide dismutase and catalase in *Drosophila melanogaster* flies that lived in hypergravity at a young age. **Biogerontology**, v. 5, n. 4, p. 261–266, 2004.

LIAO, H. et al. Science of the Total Environment Parental exposure to polystyrene nanoplastics and di (2-ethylhexyl) phthalate induces transgenerational growth and reproductive impairments through bioaccumulation in *Daphnia magna*. **Science of the Total Environment**, v. 882, n. April, p. 163-657, 2023.

MANICA-CATTANI, M. F. et al. Amazonian fruits with potential effects on covid- 19 by inflammaging modulation : a narrative review. n. 1, p. 1–12, 2022.

MIGUEL-ALIAGA, I.; JASPER, H.; LEMAITRE, B. Anatomy and physiology of the digestive tract of *drosophila melanogaster*. **Genetics**, v. 210, n. 2, p. 357–396, 2018.

MUSACHIO, E. A. S. et al. Bisphenol A exposure is involved in the development of Parkinson like disease in *Drosophila melanogaster*. **Food and Chemical Toxicology**, v. 137, n. September 2019, p. 111-128, 2020.

MUSACHIO, E. A. S. et al. Bisphenol a exposure during the embryonic period: insights into dopamine relationship and behavioral disorders in *Drosophila melanogaster*. **Food And Chemical Toxicology**, v. 157, n. august, 2021.

OHKAWA, H., OHISHI, N., YAGI, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. **Anal. Biochem.** n. 95, p. 351–358, 1979.

POETINE, M. R. et al. Chemico-Biological Interactions Hesperidin attenuates iron-induced oxidative damage and dopamine depletion in *Drosophila melanogaster* model of Parkinson ' s disease. **Chemico-Biological Interactions**, v. 279, n. November 2017, p. 177–186, 2018.

PORTELA, J. L. et al. *Ilex paraguariensis* Attenuates Changes in Mortality, Behavioral and Biochemical Parameters Associated to Methyl Malonate or Malonate Exposure in *Drosophila melanogaster*. **Neurochemical Research**, v. 44, n. 9, p. 2202–2214, 2019.

PÉREZ-SEVERIANO, F. et al. increased formation of reactive oxygen species, but no changes in glutathione peroxidase activity, in striata of mice transgenic for the huntington's disease mutation. **neurochemical research**, v. 29, n. 4, p. 729–733, 2004.

PORTELA, J. L. et al. Ilex paraguariensis Attenuates Changes in Mortality, Behavioral and Biochemical Parameters Associated to Methyl Malonate or Malonate Exposure in *Drosophila melanogaster*. **Neurochemical Research**, v. 44, n. 9, p. 2202–2214, 2019.

SOTIBRÁN, A. N. C., ORDAZ-TÉLLEZ, M. G., & RODRÍGUEZ-ARNAIZ, R. Flavonoids and oxidative stress in *Drosophila melanogaster*. **Mutation Research/Genetic Toxicology and Environmental Mutagenesis**, v. 726, n. 1, p. 60-65, 2011.

VITOROVIĆ, J., JOKOVIĆ, N., RADULOVIĆ, N., MIHAJLOV-KRSTEV, T., CVETKOVIĆ, V. J., JOVANOVIĆ, N., ... & BERNSTEIN, N. Antioxidant activity of hemp (*Cannabis sativa L.*) seed oil in *Drosophila melanogaster* larvae under non-stress and H₂O₂-induced oxidative stress conditions. **Antioxidants**, v, 10, n. 6, p. 830, 2021.

5. CONSIDERAÇÕES FINAIS

O extrato do Camu-camu atualmente está sendo consumido a livre demanda e deve-se ter uma atenção sobre sua dose diária adequada. A partir da LC50 podemos avaliar a concentração letal na fase de desenvolvimentos das moscas, e durante os experimentos realizados analisamos que as maiores quantidades de concentração do extrato adquirem um efeito negativo em relação a atividade antioxidante, ou seja, alterando os parâmetros de estresse oxidativo. Como também nas enzimas antioxidantes que resultaram nos testes comportamentais danos neurológicos nas moscas. Deve-se levar em consideração que a atividade pró-oxidante em um organismo vivo pode resultar em várias doenças crônicas que se em grandes quantidades de espécies reativas, os danos podem ser permanentes, semelhante ao resultado desse estudo, no qual houve morte das larvas, interrompendo a formação da pupa e em seguida na eclosão da mosca.

Utilizamos a concentração de 0,5mg/mL pois foi a concentração no qual as análises realizadas não apresentaram danos oxidativos nas larvas das moscas, sendo a partir da quantidade de 5mg/mL que pôde ser observado o possível dano (em mortalidade, locomotor e pró-oxidante) que essa concentração pode ocasionar neste modelo experimental. A partir dos resultados adquiridos durante o estudo em exposição ao Fe para analisarmos possível efeito protetor do extrato. Durante os experimentos as moscas apresentaram resistência aos danos oxidativos, no qual mostra que o extrato do fruto apresenta efeito protetor. A partir disso, conclui-se na eficácia do Camu-camu ao estresse oxidativo e os danos que o mesmo pode causar em um organismo vivo, como na literatura ainda não há estudos semelhantes, se avalia a necessidade de mais estudos pilotos para comprovar a eficiência e os efeitos do extrato do fruto Camu-camu.

6. REFERÊNCIAS

- AZEVEDO, L. et al. camu-camu (*myrciaria dubia*) from commercial cultivation has higher levels of bioactive compounds than native cultivation (amazon forest) and presents antimutagenic effects in vivo. **journal of the science of food and agriculture**, v. 99, n. 2, p. 624–631, 2019.
- BEHL, T. et al. Mechanistic Insights Expatriating the Redox-Active-Metal-Mediated Neuronal Degeneration in Parkinson's Disease. **International Journal of Molecular Sciences**, v. 23, n. 2, p. 1–26, 2022.
- BOOTH, L. N.; BRUNET, A. The Aging Epigenome. **Molecular Cell**, v. 62, n. 5, p. 728–744, 2016.
- CAVALIERI, I. Cor e gosto das fruteiras. **Manchete Rural**, v. 72, n. 6, p. 28-31, 1993.
- COLD, D. E. The relationship between cerebral metabolic rate of oxygen and cerebral blood flow in the acute phase of head injury. **Acta Anaesthesiologica Scandinavica**. v. 30, p. 453-457, 1986.
- CHEDRAUI, P.; PÉREZ-LÓPEZ, F. R. Nutrition and health during mid-life: Searching for solutions and meeting challenges for the aging population. **Climacteric**, v. 16, n. S1, p. 85–95, 2013.
- DAY, B. J. antioxidant therapeutics: pandoras box. **free radical biology and medicine**, v. 66, p. 58–64, 2014.
- DELGADO-LISTA, J. Long-term Secondary Prevention of Cardiovascular Disease with a Mediterranean diet and a Low-Fat diet, The CORDIOPREV Study: a randomized clinical trial. v. 1, p. 1–28, 2022.
- DO CARMO, M. A. V. et al. camu-camu (*Myrciaria dubia*) seeds as a novel source of bioactive compounds with promising antimalarial and antischistosomicidal properties. **food research international**, v. 136, n. february, p. 109-334, 2020.
- DO, N. Q. et al. anti-allergic effects of *Myrciaria dubia* (camu-camu) fruit extract by inhibiting histamine h1 and h4 receptors and histidine decarboxylase in rbl-2h3 cells. **antioxidants**, v. 11, n. 1, 2022.
- DOMINGUEZ, L. J. et al. Impact of mediterranean diet on chronic non-communicable diseases and longevity. **Nutrients**, v. 13, n. 6, p. 1–31, 2021.
- DUFOUR, D. L. et al. Amazonian foods and implications for human biology. **Annals of Human Biology**, v. 43, n. 4, p. 330–348, 2016.
- FORMAN, H. J.; ZHANG, H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. **nature reviews drug discovery**, v. 20, n. 9, p. 689–709, 2021.
- FRACASSETTI, D. et al. Ellagic acid derivatives, ellagitannins, proanthocyanidins and other phenolics, vitamin C and antioxidant capacity of two powder products from camu-camu fruit (*Myrciaria dubia*). **Food chemistry**, v. 139, n. 1-4, p. 578-588, 2013.

FRANCO, M. R. B.; JANZANTTI, N. S. Aroma of minor tropical fruits. **Flavour and fragrance journal**, v. 20, n. 4, p. 358-371, 2005.

GARCÍA-CHACÓN, J. M.; MARÍN-LOAIZA, J. C.; OSORIO, C. Camu Camu (*Myrciaria dubia* (Kunth) McVaugh): An Amazonian Fruit with Biofunctional Properties-A Review. **ACS Omega**, v. 8, n. 6, p. 5169–5183, 2023.

HARMAN, D. The aging process. **Proceedings of the National Academy of Sciences of the United States of America**, v. 78, n. 11, p. 7124–7128, 1981.

KENNEDY, B. K. et al. Aging: a common driver of chronic diseases and a target for novel interventions. **Cell**, v. 159, n. 4, p. 709, 2014.

LANGLEY, P. C. et al. antioxidant and associated capacities of camu camu (*Myrciaria dubia*): a systematic review. *journal of alternative and complementary medicine*, v. 21, n. 1, p. 8–14, 2015.

LIGUORI, I. et al. Oxidative Stress and Diseases. **Oxidative Stress and Diseases**, v. 13, p. 757–772, 2012.

LIMA, R. S.; DE CARVALHO, A. P. A.; CONTE-JUNIOR, C. A. Health from Brazilian Amazon food wastes: Bioactive compounds, antioxidants, antimicrobials, and potentials against cancer and oral diseases. *Critical Reviews in Food Science and Nutrition*, p. 1-23, 2022.

LOCKE, A.; SCHNEIDERHAN, J.; ZICK, S. M. Diets for health: goals and guidelines. **American family physician**, v. 97, n. 11, p. 721-728, 2018.

LUO, J. et al. Ageing, age-related diseases and oxidative stress: what to do next? ageing research reviews, v. 57, p. 100-982, 2020.

MACIEL, V. B. DA S. et al. Food diversity among indigenous children from two municipalities of the brazilian western amazon. **Ciencia e Saude Coletiva**, v. 26, n. 7, p. 2921–2928, 2021.

MCMACKEN, M.; SHAH, S. A plant-based diet for the prevention and treatment of type 2 diabetes. **Journal of Geriatric Cardiology**, v. 14, n. 5, p. 342–354, 2017.

MIGUEL-ALIAGA, I.; JASPER, H.; LEMAITRE, B. Anatomy and physiology of the digestive tract of *Drosophila melanogaster*. **Genetics**, v. 210, n. 2, p. 357–396, 2018.

MIRANDA, A.; GOMEZ-GAETE, C.; MENNICKENT, S. Role of Mediterranean diet on the prevention of Alzheimer disease. **Revista medica de Chile**, v. 145, n. 4, p. 501-507, 2017.

MUSACHIO, E. A. S. et al. Bisphenol a exposure during the embryonic period: insights into dopamine relationship and behavioral disorders in *Drosophila melanogaster*. **Food And Chemical Toxicology**, v. 157, n. august, 2021.

NASCIMENTO, O. V. et al. Effects of diet supplementation with camu-camu (*Myrciaria dubia hbk mcvaugh*) fruit in a rat model of diet-induced obesity. **Anais Da Academia Brasileira De Ciencias**, v. 85, n. 1, p. 355–363, 2013.

NERI-NUMA, I. A. et al. Small brazilian wild fruits: nutrients, bioactive compounds, health-promotion properties and commercial interest. **Food Research International**, v. 103, p. 345–360, 2018.

OZEMEK, C. et al. The role of diet for prevention and management of hypertension. **Current Opinion in Cardiology**, v. 33, n. 4, p. 388–393, 2018.

PARDO-ALDAVE, K.; PAREJA-VÁSQUEZ, M.; GUILLÉN, A.U.T. J. Artículo original antimicrobial activity in vitro of camu-camu (*Myrciaria dubia*). **Rev Peru Med Exp Salud Publica**, v. 36, n. 4, p. 573–582, 2019.

PRIOR, R. L.; CAO, G. Antioxidant Phytochemicals in Fruits and Vegetables: Diet and Health Implications. **HortScience**. v. 35, p. 588-592, 2000.

RIBEIRO, E. E.; DA CRUZ, I. B. M. **Dieta Amazônica - Saúde e Longevidade**. Editora Cultural da Amazônia, 2010.

SALAS-SALVADÓ, J. et al. Mediterranean Diet and Cardiovascular Disease Prevention: What Do We Know? **Progress in Cardiovascular Diseases**, v. 61, n. 1, p. 62–67, 2018.

SENONER, T.; DICHTL, W. Oxidative stress in cardiovascular diseases: still a therapeutic target? nutrients, v. 11, n. 9, 2019.

SHAHIDI, F.; AMBIGAIPALAN, P. Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects - a review. **Journal Of Functional Foods**, v. 18, p. 820–897, 2015.

SIES, H. Oxidative stress: a concept in redox biology and medicine. **Redox Biology**, v. 4, p. 180–183, 2015.

SOSA, R. et al. Antioxidant Properties of Amazonian Fruits: A Mini Review of *in vivo* and in Vitro Studies. **Oxidative Medicine and Cellular Longevity**, v. 2019, 2019.

STAATS, S. et al. *Drosophila melanogaster* as a versatile model organism in food and nutrition research. **Journal Of Agricultural And Food Chemistry**, v. 66, n. 15, p. 3737–3753, 2018.

TAN, B. L.; NORHAIZAN, M. E.; LIEW, W. P. P. Nutrients and oxidative stress: friend or foe? **Oxidative Medicine And Cellular Longevity**, 2018.

TRÉZ, T. DE A. E. Considerações sobre o conceito dos 3rs e o potencial conflito com novas compreensões do animal experimental. **revista brasileira de zootecias**, v. 19, n. 2, p. 97–113, 2018.

TROHA, K.; BUCHON, N. Methods for the study of innate immunity in drosophila melanogaster. **wiley interdisciplinary reviews: developmental biology**, v. 8, n. 5, p. 1–25, 2019.

WYATT, L., et al. Spatial, temporal, and dietary variables associated with elevated mercury exposure in Peruvian riverine communities upstream and downstream of artisanal and small-scale gold mining. **International Journal of Environmental Research and Public Health**, v. 14, n. 12, p. 1582, 2017.

YAMAGUCHI, M.; YOSHIDA, H. *Drosophila* as a model organism. advances in experimental medicine and biology, v. 1076, p. 1–10, 2018.