UNIVERSIDADE FEDERAL DO PAMPA

PROGRAMA DE PÓS-GRADUÇÃO EM BIOQUÍMICA

ANDRÉIA LIMANA TAMBARA

POTENCIAL ANTIOXIDANTE E ANTIOBESIDADE DE FRUTAS NATIVAS DO SUL DO BRASIL UTILIZANDO O MODELO *Caenorhabditis elegans*

URUGUAIANA

2022

ANDRÉIA LIMANA TAMBARA

POTENCIAL ANTIOXIDANTE E ANTIOBESIDADE DE FRUTAS NATIVAS DO SUL DO BRASIL UTILIZANDO O MODELO *Caenorhabditis elegans*

Tese apresentada ao Programa de Pós-graduação Stricto Sensu em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do título de Doutora em Bioquímica.

Orientadora: Cristiane Casagrande Denardin

URUGUAIANA
2022

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) .

T154p Tambara, Andréia Limana
Potencial antioxidante e antiobesidade de frutas nativas do sul do Brasil utilizando o modelo Caenorhabditis elegans / Andréia Limana Tambara.
101 p.

Tese(Doutorado)-- Universidade Federal do Pampa, DOUTORADO EM BIOQUÍMICA, 2023.
"Orientação: Cristiane Casagrande Denardin".

1. butiá . 2. pitanga. 3. estresse oxidativo. 4. triglicerídeos. 5. C. elegans. I. Título.

ANDRÉIA LIMANA TAMBARA

POTENCIAL ANTIOXIDANTE E ANTIOBESIDADE DE FRUTAS NATIVAS DO SUL DO BRASIL UTILIZANDO O MODELO *Caenorhabditis elegans*

Tese apresentada ao Programa de Pós-graduação Stricto Sensu em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do título de Doutora em Bioquímica.

Tese defendida e aprovada em: 20 de Janeiro de 2023.

Banca examinadora:

_____

Profa. Dra. Cristiane Casagrande Denardin

Orientadora

UNIPAMPA

_____

Profa. Dra. Fatima Theresinha Costa Rodrigues Guma

UFRGS

_____

Profa. Dra. Greicy Michelle Marafiga Conterato

UFSC

_____

Profa. Dra. Jacqueline da Costa Escobar Piccoli

UNIPAMPA

_____

Profa. Dra. Sandra Elisa Haas

UNIPAMPA

Dedico este trabalho a minha querida avó Ângela (*in memoriam*).

AGRADECIMENTO

Agradeço primeiramente a Deus e a meus pais, Enio e Láutia, obrigada pelo apoio de vocês em todos os momentos dessa minha jornada, amo muito vocês.

Ao meu irmão André por sempre estar presente e me incentivando a estudar.

A meu noivo Luís Fernando pelo apoio emocional ao longo desses anos.

A minha querida orientadora a Professora Dra. Cristiane, que me passou muita sabedoria durante toda minha formação.

A todos os colegas do meu Grupo de Pesquisa em Bioquímica e Toxicologia de Compostos Bioativos (GBToxBio).

A todos os colegas do Grupo de Pesquisa em Bioquímica e Toxicologia em *Caenorhabdits elegans* (GBToxCe).

A Universidade Federal do Pampa (Unipampa) por toda minha formação acadêmica.

Agradeço a todos que de alguma forma me auxiliaram para conclusão desse trabalho.

“A chave para realizar um sonho é se concentrar não no sucesso, mas em um propósito e, em seguida, até mesmo os pequenos passos e pequenas vitórias ao longo do seu caminho vão assumir um significado maior”.

Oprah Winfrey

RESUMO

Recentemente, o interesse no consumo de frutos nativos vem crescendo, em razão do potencial benéfico à saúde humana que apresentam, atribuído à presença de substâncias antioxidantes como os compostos fenólicos, as vitaminas e os minerais que contribuem na prevenção de muitas doenças. O butiá (*Butia eriospatha*) é um fruto originário de uma palmeira pertencente à família *Arecaceae*, nativa da América do Sul e a pitanga é um fruto da pitangueira (*Eugenia uniflora* L.), pertence à família botânica *Myrtaceae*, é uma planta frutífera nativa do Brasil. Este trabalho tem como objetivo avaliar o potencial antioxidante do extrato da fruta butiá e o potencial antiobesidade do extrato da fruta pitanga roxa utilizando o modelo experimental *Caenorhabditis elegans*. Inicialmente, foi realizado experimentos de sobrevivência, reprodução, resistência ao estresse oxidativo, longevidade, expressão de superóxido dismutase e catalase com o extrato etanólico da polpa da fruta butiá. Observamos que o extrato de butiá não afetou a sobrevivência e a postura de ovos dos vermes, foi capaz de reverter o dano oxidativo induzido pelo peróxido de hidrogênio. Além disso, o extrato de butiá aumentou a expectativa de vida de *C. elegans* sob estresse. Também foi realizada a padronização de um modelo de indução da obesidade pela dieta utilizando lipossomas com colesterol em *C. elegans*. E quando os vermes selvagens foram tratados com o extrato etanólico da fruta pitanga roxa, após o cultivo em meio com alto teor de colesterol, os vermes apresentaram níveis diminuídos de triglicerídeos, colesterol, lipídios totais e glicose, além de um aumento da longevidade. No ensaio de consumo alimentar não observamos alteração significativa na ingestão alimentar. Os níveis de triglicerídeos na cepa RB1600 (mutante *tub-1*) também apresentaram diminuição. Os resultados demonstram que o extrato de butiá é capaz de prolongar o tempo de vida do nematoide *C. elegans* e que esse efeito pode ser mediado por uma resistência induzida ao estresse oxidativo. Além disso, os resultados indicam que o extrato da fruta pitanga roxa desempenha um papel importante na redução da deposição de gordura, modulando as vias celulares para o acúmulo de lipídios e prolongando a vida útil do *C. elegans*.

Palavras-chave: butiá; pitanga; estresse oxidativo; triglicerídeos; *C. elegans*.

ABSTRACT

Recently, interest in the consumption of native fruits has been growing, due to the beneficial potential for human health that they present, attributed to the presence of antioxidant substances such as phenolic compounds, vitamins and minerals that contribute to the prevention of many diseases. The butiá (*Butia eriospatha*) is a fruit originating from a palm tree belonging to the *Arecaceae* family, native to South America and the pitanga is a fruit of the pitangueira (*Eugenia uniflora* L.), belongs to the botanical family *Myrtaceae*, is a fruit plant native to the Brazil. This work aims to evaluate the antioxidant potential of the butiá fruit extract and the anti-obesity potential of the purple pitanga fruit extract using the experimental model *Caenorhabditis elegans*. Initially, experiments were carried out on survival, reproduction, resistance to oxidative stress, longevity, superoxide dismutase and catalase expression with the ethanolic extract of butiá fruit pulp. We observed that butiá extract did not affect the survival and egg laying of the worms, it was able to reverse the oxidative damage induced by hydrogen peroxide. Furthermore, butia extract increased the life expectancy of *C. elegans* under stress. The standardization of a model of induction of obesity by diet using liposomes with cholesterol in *C. elegans* was also carried out. And when the wild worms were treated with the ethanolic extract of the purple pitanga fruit, after cultivation in a medium with a high cholesterol content, the worms showed decreased levels of triglycerides, cholesterol, total lipids and glucose, in addition to an increase in longevity. In the food consumption test, we did not observe any significant change in food intake. Triglyceride levels in the RB1600 strain (*tub-1* mutant) also decreased. The results demonstrate that the butiá extract is capable of prolonging the lifespan of the nematode *C. elegans* and that this effect may be mediated by an induced resistance to oxidative stress. Furthermore, the results indicate that the purple pitanga fruit extract plays an important role in reducing fat deposition, modulating cellular pathways for lipid accumulation and prolonging the lifespan of *C. elegans*.

Keywords: butiá; pitanga; oxidative stress; triglycerides; *C. elegans*.

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

1 INTRODUÇÃO

As frutas e os vegetais fazem parte do grupo de alimentos mais importantes no controle do peso e prevenção de doenças (Singh, 2004; Knai *et al.*, 2006) e o consumo adequado tem sido associado com risco diminuído de doenças crônicas (Dauchet *et al.*, 2006; Prynne *et al.*, 2006). Algumas plantas medicinais possuem um potencial hipolipemiante, e a maioria dos extratos de plantas apresentam uma atividade antioxidante (Pizziolo *et al.*, 2011). Há um crescente interesse em encontrar antioxidantes naturais, em especial de origem vegetal, a serem utilizados no tratamento de várias doenças humanas. Suas propriedades farmacológicas e terapêuticas têm sido atribuídas a diferentes constituintes químicos isolados de seus extratos, no entanto, alguns estudos demonstraram que os extratos têm uma eficácia mais elevada, quando comparada com as moléculas isoladas (Surveswaran *et al.*, 2010).

O Butiá (*Butia eriospatha*) popularmente conhecido por coquinho-azedo, é um fruto originário de uma palmeira pertencente a família *Arecaceae*, nativa da América do Sul, que possui frutos comestíveis com sementes utilizadas como oleaginosas. No Brasil, ocorrem de forma endêmica e naturalmente em áreas abertas e nas florestas com a Araucária do planalto sul dos estados do Paraná, Santa Catarina e Rio Grande do Sul. Os frutos maduros podem ser consumidos *in natura* ou usados na elaboração de sucos, vinhos e licores (Henderson et. al. 1995).

A pitanga (também conhecida por “Brazilian cherry”), fruto da pitangueira (*Eugenia uniflora* L.) é originária do Brasil, espalhando-se desde o nordeste até o Rio Grande do Sul, ultrapassando fronteiras para chegar a algumas regiões do Uruguai e da Argentina (Bezerra, 2000). Com relação às conhecidas atividades terapêuticas da pitangueira, suas folhas, têm sido referenciadas como eficientes no tratamento de diversas enfermidades com ação antiinflamatória (Schapoval *et al.*, 1994), para tratamento da bronquite (Rivera e Obon, 1995), com ação calmante (Grainger, 1996), com atividade diurética (Consolini *et al.*, 1999), para tratamento da diabetes e obesidade (Arai *et al.*, 1999), com atividade cardiovascular (Lee *et al.*, 2000) e antioxidante (Velazquez *et al.*, 2003). Já o extrato dos frutos apresentou efeito antioxidante em *C. elegans* (Tambara *et al.*, 2018) e alguns efeitos preliminares na redução do acúmulo de lipídios em cultura celular (Denardin *et al.*, 2014).

Para avaliar os efeitos dos extratos das frutas butiá e pitanga roxa *in vivo*, foi utilizado o modelo experimental *Caenorhabditis elegans* que é um pequeno nematoide, que tornou-se um organismo modelo proeminente para um grande número de estudos de desenvolvimento biológico, comportamento, envelhecimento, ecotoxicológicos e genéticos (Diogo e Mota, 2001). Esses fatores fazem com que este organismo seja a escolha ideal para o estudo.

Muitas pesquisas vêm sendo feitas na busca de agentes naturais, com o objetivo de promover um maior conhecimento sobre os compostos com propriedades benéficas presentes nestes vegetais que podem atuar na prevenção ou tratamento de enfermidades. Tendo em vista o exposto acima, nossa hipótese é que o extrato da fruta butiá apresente um potencial antioxidante e o extrato da fruta pitanga roxa possua efeito sobre o acúmulo de lipídios no modelo *C. elegans*.

2 REVISÃO DE LITERATURA

2.1 Estresse oxidativo e antioxidantes

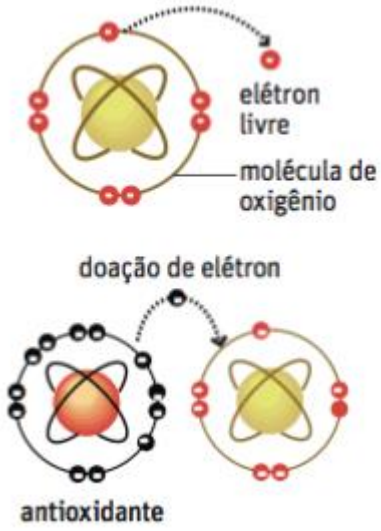
A geração de radicais livres constitui um processo contínuo e fisiológico, cumprindo funções biológicas relevantes. Define-se radicais livres ou espécies reativas (RLs) espécies independentes que contêm um ou mais elétrons não pareados. A presença de elétrons não pareados no átomo ou na molécula aumenta a sua reatividade química. Além disso, essa característica confere-lhes grande instabilidade, por tenderem a acoplar o elétron não pareado com um outro que esteja presente em estruturas próximas à sua formação. Os mecanismos de geração de radicais livres ocorrem, normalmente, nas mitocôndrias, membranas celulares e no citoplasma. A mitocôndria, por meio da cadeia transportadora de elétrons, é a principal fonte geradora de radicais livres. Espécies reativas de oxigênio (EROs), tais como radical hidroxila (•OH), ânion radical superóxido (O2•–) e hidroperoxila (ROO•), causam danos ao DNA ou podem oxidar lipídios e proteínas (Leite e Sarni, 2003; Barbosa *et al.*, 2010; Vellosa *et al.*, 2021).

A instalação do processo de estresse oxidativo decorre da existência de um desequilíbrio entre compostos oxidantes e antioxidantes, em favor da geração excessiva de radicais livres ou em detrimento da velocidade de remoção desses. Tal processo conduz à oxidação de biomoléculas com consequente perda de suas funções biológicas e/ou desequilíbrio homeostático, cuja manifestação é o dano oxidativo potencial contra células e tecidos. A cronicidade do processo em questão tem relevantes implicações sobre o processo de numerosas enfermidades crônicas, entre elas a aterosclerose, diabetes, obesidade, transtornos neurodegenerativos e câncer (Halliwell e Whiteman, 2004; Barbosa *et al.*, 2010).

A produção contínua de radicais livres durante os processos metabólicos culminou no desenvolvimento de mecanismos de defesa antioxidante. Estes têm o objetivo de limitar os níveis intracelulares de tais espécies reativas e controlar a ocorrência de danos decorrentes. O sistema de defesa antioxidante tem a função de inibir e/ou reduzir os danos causados pela ação deletéria dos radicais livres ou das espécies reativas não-radicais (Barbosa *et al.*, 2010). A produção de radicais livres é controlada nos seres vivos por diversos compostos antioxidantes, os quais podem ter origem endógena, ou serem provenientes da dieta alimentar e outras fontes (Sousa *et al.*, 2007).

Os antioxidantes são definidos como qualquer substância que, presente em menores concentrações que as do substrato oxidável, seja capaz de atrasar ou inibir a oxidação deste de maneira eficaz (Halliwell e Whiteman, 2004). Tais substâncias podem agir diretamente, neutralizando a ação dos radicais livres e espécies não-radicais como representado na Figura 1, ou indiretamente, participando dos sistemas enzimáticos com tal capacidade.

Figura 1: Radical livre e antioxidante



Fonte: http://radicaislivreseantioxidantes.blogspot.com.br/2014/

O sistema de defesa enzimático inclui as enzimas superóxido dismutase (SOD), catalase (CAT), glutationa peroxidase (GPx) e glutationa redutase (GR) (Barbosa *et al.*, 2010; Vellosa *et al.*, 2021). As células aeróbias são protegidas da ação do superóxido e do peróxido de hidrogênio pela ação da superóxido-dismutase (SOD), uma metaloenzima que converte o radical superóxido em peróxido de hidrogênio, e pela ação da catalase, que converte o peróxido de hidrogênio em água e oxigênio molecular. A SOD citoplasmática necessita da presença de cobre e zinco para agir e a mitocondrial, de manganês; já a catalase depende do ferro. Além da catalase, outra enzima importante no controle dos peróxidos é a glutationa peroxidase (GPx), que utiliza para sua ação a glutationa (GSH), um tripeptídeo contendo cisteína, e representa o tiol não proteico mais abundante nas células de mamíferos. É substrato para as enzimas antioxidantes: *GSH transferases e peroxidases* (dependentes de selênio). As demais funções da GSH envolvem sua participação no estoque e

transporte de cisteína, regulação do balanço "redox", metabolismo de prostaglandinas e leucotrienos, síntese de desoxirribonucleotídeos, função imune e proliferação celular (Leite e Sarni, 2003). Essas enzimas agem por meio de mecanismos de prevenção, impedindo e/ou controlando a formação de radicais livres e espécies não-radicais, envolvidos com a iniciação das reações em cadeia que culminam com propagação e amplificação do processo e, consequentemente, com a ocorrência de danos oxidativos (Barbosa *et al.*, 2010).

O sistema de defesa não enzimático inclui, especialmente, os compostos antioxidantes de origem dietética, entre os quais se destacam: vitaminas e compostos fenólicos (Barbosa *et al.*, 2010). A atividade antioxidante de compostos fenólicos deve-se principalmente às suas propriedades redutoras e estrutura química. Estas características desempenham um papel importante na neutralização ou sequestro de radicais livres e quelação de metais de transição, agindo tanto na etapa de iniciação como na propagação do processo oxidativo. Os carotenos protegem os lipídios dos danos oxidativo, através da reação com os radicais peroxila, hidroxila e superóxido. A atividade antioxidante dos carotenoides é decorrente da habilidade de sequestrar espécies reativas de oxigênio devido à presença de ligações duplas conjugadas em sua estrutura (Sousa *et al.*, 2007).

Os antioxidantes presentes nas frutas atuam como agentes redutores de espécies reativas de oxigênio, razão pela qual o consumo de alimentos que possuem essa característica é importante (De Souza *et al.*, 2018).

2.2 Consumo de frutas

A alimentação e a nutrição adequadas constituem-se em requisitos básicos para a promoção e a proteção da saúde e para o desenvolvimento sustentável (Haddad *et al.*, 2014). O consumo de frutas e hortaliças tem aumentado principalmente em decorrência do seu valor nutritivo e efeitos terapêuticos. Esse tem sido associado à diminuição do risco de mortalidade (Agudo *et al.*, 2007) e redução da ocorrência de doenças crônicas, tais como as doenças cardiovasculares (Dauchet *et al.*, 2006), derrames (He *et al.*, 2006) e alguns tipos de câncer (Key *et al.*, 2002). A Organização Mundial da Saúde (OMS) afirma que existem evidências convincentes

de que o consumo de frutas, legumes e verduras também diminui o risco de diabetes e obesidade. Eles também recomendam um consumo mínimo de pelo menos cinco porções diárias de frutas, legumes e verduras, o que equivale a 400g ou mais por dia (Who e Consultation, 2003).

Embora o Brasil seja um grande produtor mundial de frutas e hortaliças, com grande abundância de variedades nas diferentes regiões do país, o brasileiro ainda consume pouco esses alimentos, há várias pesquisas que demonstram o baixo consumo pela população (Vigitel, 2018). Dados de consumo, entretanto, mostram ingestão insuficiente em nível mundial e em todos os grupos etários. Observa-se uma prevalência de consumo abaixo das recomendações em 90% da população e existem evidências de que a maioria sequer conhece as recomendações para estes alimentos (Machado *et al.*, 2016).

A inadequação do consumo de frutas e hortaliças possui abordagem complexa. Ocorre por questões ambientais, como sistemas de distribuição e comercialização; econômicos, como preços elevados comparados a outros alimentos; e individuais (Who, 2004). Entre os fatores individuais está a baixa renda, ser solteiro e possuir comportamentos não saudáveis, como tabagismo, sedentarismo e alimentação rica em açúcar e gorduras. Por outro lado, o consumo de frutas e hortaliças parece ser maior com o aumento da escolaridade e da idade dos indivíduos (Figueiredo *et al.*, 2008; Jaime *et al.*, 2009; Neutzling *et al.*, 2009; Campos *et al.*, 2010; Mondini *et al.*, 2010; Nepal *et al.*, 2012).

Portanto, o incentivo ao consumo e produção de frutas pode ser melhorado com pesquisas que promovam um maior conhecimento sobre os compostos com propriedades benéficas presentes nestes vegetais que podem atuar na prevenção ou tratamento de enfermidades. As frutas e vegetais contêm diferentes fitoquímicos, muitos dos quais possuem propriedades antioxidantes que pode estar relacionada com o retardo do envelhecimento e a prevenção de certas doenças (Wang e Prior, 1996). A atividade antioxidante apresentada por vários vegetais, incluindo frutos, folhas, sementes e plantas medicinas, está correlacionada ao seu teor de compostos fenólicos totais (Velioglu *et al.*, 1998). Estudos mostram que uma variedade de produtos naturais, incluindo extratos e compostos isolados de plantas, estão sendo utilizados para a redução do peso corporal e prevenir a obesidade induzida por dieta hipercalórica (Moro e Basile, 2000; Hanl *et al.*, 2005; Rayalam *et al.*, 2008).

Frutos de espécies nativas do Brasil têm despertado a atenção da população pelas suas características nutracêuticas (Silveira *et al.*, 2005). O interesse nestas espécies nativas vem crescendo nos últimos anos devido aos possíveis benefícios que podem proporcionar à saúde humana, o que vem incentivando o desenvolvimento de pesquisas sobre as suas características nutricionais e de qualidade dos frutos. Os benefícios à saúde atribuídos aos alimentos naturalmente ricos em compostos fenólicos e outros antioxidantes têm elevado a procura por novas espécies botânicas que possuam, além dessa propriedade, atividades biológicas complementares relevantes (Céspedes *et al.*, 2008)

2.3 Butiá (*Butia eriospatha*)

As espécies pertencentes à família *Palmae* ou *Aracaceae*, comumente chamadas de palmeiras, são muito atrativas do ponto de vista químico e farmacológico (Silveira et al., 2005). A família *Palmae* apresenta distribuição diversificada na Ásia Tropical e na América do Sul, incluindo de 3.000-3.700 espécies distribuídas em aproximadamente 240-387 gêneros (Bora et al., 2003). No Rio Grande do Sul ocorrem seis gêneros de palmeiras nativas: *Bactris* Jacq., *Butia* (Mat.) Becc., *Euterpe* Mart., *Geonoma* Mart., *Syagrus* Mart. e *Trithrinax* Mart. Cada gênero ocupa um bioma específico, mas dificilmente ocorrem interações entre as diferentes espécies (Rossato, 2007). Entre eles, destacam-se as palmeiras do gênero *Butia* que é um dos gêneros da subtribo *Buttinae*, tribo *Cocoeae*, subfamília *Arecoideae*. O Butiazeiro (Figura 2) pertence a um pequeno gênero de palmeiras subtropicais com distribuição no sul da América do Sul, ocorrendo naturalmente no sul do Brasil, leste do Paraguai, nordeste da Argentina e no noroeste e sudeste do Uruguai (Tonietto et al., 2009).

Figura 2: Butiazeiro (*Butia eriospatha*)

Fonte: Espécies Arbóreas Brasileiras – Emprapa

Especificamente do gênero *Butia*, no Rio Grande do Sul os registros de maior ocorrência são de *B*. *capitata* e *B. eriospatha* (Rossato et al., 2007). Estas palmeiras produzem frutos comestíveis na forma *in natura* ou podem ser utilizados para o preparo de sucos, licores e sorvetes. Seus frutos são muito explorados pela população devido ao sabor e ao aroma intenso e peculiar. O fruto butiá (Figura 3) também conhecido como coquinho-azedo apresenta tamanho pequeno (com diâmetro médio

de 1,7 a 1,9 cm), podendo ser enquadrado como pequeno fruto, sua maturação geralmente ocorre de novembro a maio, tendo seu pico no verão, no mês de fevereiro (Rosa et al., 1998). O interesse por estas espécies nativas e pequenos frutos vem crescendo nos últimos anos devido aos benefícios que podem proporcionar à saúde humana, que vem incentivar o desenvolvimento de pesquisas sobre as características nutricionais e qualidade dos frutos para que sejam destinados ao consumo sob forma *in natura* ou para o processamento de derivados.

Figura 3: Butiá (*Butia eriospatha*)

Fonte: Espécies Arbóreas Brasileiras – Emprapa

As espécies de butiás *Butia capitata* e *Butia eriosphata* foram citadas como integrantes da lista da flora medicinal do Rio Grande do Sul devido a benefícios medicinais relatados pela população (Mentz *et al.*, 1997). Na medicina popular, o chá da flor do butiá é indicado no combate ao amarelão e como calmante, para equilibrar o sono. Ainda, segundo os saberes dessa medicina, a polpa do fruto, consumida *in natura*, ajuda a eliminar o ácido úrico (Carvalho, 2014). A composição da polpa do butiá está apresentada na Tabela 1.

Tabela 1: Composição centesimal e composição em fitoquímicos da polpa de frutos de butiá (*B. eriosphata*).

Componente	**Unidade**	**Valor**
Valor energético	cal	42,30
Umidade	%	88,15
Proteína	%	1,07
Lipídios	%	0,15
Carboidratos	%	9,16
Fibra	%	0,88
Cinza	%	0,59
Vitamina C	mg	21,34
Antocianinas totais	mg	0,73
Carotenoides totais	mg	17,27
Fenóis totais	mg	278,38

Fonte: (Sganzerla, 2010).

A polpa do butiá apresenta quantidades consideráveis de vitamina C, carotenoides e compostos fenólicos, componentes considerados importantes antioxidantes naturais. Devido ao seu valor nutricional e abundância, o consumo desta fruta deve ser incentivado, pois é fonte de nutrientes e compostos bioativos, importantes para o crescimento, desenvolvimento e proteção contra doenças (Sganzerla, 2010; Denardin *et al.*, 2015; Barbosa *et al.*, 2021).

2.4 Pitanga (*Eugenia uniflora* L.)

Segundo classificação de Cronquist (Cronquist, 1988) a pitangueira pertence à classe Magnoliopsida, subclasse Rosidae, ordem Myrtales, família Myrtaceae, gênero *Eugenia* e espécie *Eugenia uniflora* L. A pitangueira é uma frutífera de ampla distribuição geográfica. É nativa do Brasil e encontra-se disseminada, praticamente, por todo o território nacional. Em função da adaptação às diferentes condições de solo e clima, a pitangueira é encontrada em diversas partes do mundo. Há registros de cultivos em outros países da América do Sul e Central, no Caribe, nos Estados Unidos (Flórida, Califórnia, Havaí), China, Índia, Sri Lanka, México, Madagascar, África do Sul, Israel e vários países do Mediterrâneo (Popenoe, 1920; Moreuil, 1971; Sturrock, 1972; Correa e Penna, 1974; Campbell, 1977; Fouqué, 1981; Lahav e Slor, 1997).

A árvore é pequena, mede entre 2 a 4 m de altura, o tronco geralmente é tortuoso e com muitos galhos, possui folhas pequenas e verde-escuras, que exalam aroma característico, as flores são brancas e perfumadas. O fruto da pitanga como representado na Figura 4 é uma baga globosa, com sete a dez sulcos longitudinais de 1,5 a 5,0 cm de diâmetro, coroado com sépalas persistentes e aproximadamente 66% do fruto é constituído de polpa que possui aroma característico intenso e sabor doce e ácido, pode ser consumida fresca ou processada, na indústria os frutos são utilizados na produção de suco, polpa congelada e sorvetes, assim como na indústria de cosméticos. Em decorrência de uma ampla diversidade genética, a pitanga apresenta cor que varia do laranja, passando pelo vermelho, e chegando ao roxo, ou quase preto (Bezerra, 2000; Silva, 2006). Durante a maturação, o epicarpo da fruta evolui de verde para laranja e vermelho, nas variedades laranja e vermelha e, do verde ao roxo profundo ou quase preto, na variedade roxa como representado na Figura 5.

Figura 4: Pitanga roxa

Fonte: http://stravaganzastravaganza.blogspot.com.br/2012/08/alimentos-que-curam.html

Figura 5: Todas as variedades da pitanga

Fonte: http://ciprest.blogspot.com.br/2016/09/pitanga-tutti-colore-eugenia-uniflora.html

Na Tabela 2 encontram-se os valores referentes à composição média de 100g de polpa de pitanga.

Tabela 2: Valor nutricional de 100 g de polpa de frutos de pitangueira.

Componente	**Unidade**	**Valor**
Valor energético	*Cal*	51,00
Umidade	g	85,80
Proteína	g	0,80
Gordura	g	0,40
Carboidratos	g	12,50
Fibra	g	0,60
Cinza	*g*	0,50
Vitamina A	mg	635,00
Tiamina	mg	0,30
Riboflavina	mg	0,60
Niacina	mg	0,30
Ácido ascórbico	mg	14,00
Cálcio	mg	9,00
Fósforo	mg	11,00
Ferro	mg	0,20

Fonte: (De Lira Júnior *et al.*, 2007)

O crescente interesse por estas frutas está relacionado com as grandes quantidades de catequinas, flavonoides, pro-antocianidinas e compostos fenólicos, conhecidos por sua atividade antioxidante, que elas podem apresentar (Bagetti *et al.*, 2011; Celli *et al.*, 2011). Além dos diversos efeitos na saúde humana, os compostos antioxidantes são também importantes na inibição e/ou prevenção da oxidação de produtos alimentares. As folhas da pitangueira têm sido utilizados a muito tempo na medicina popular, devido a suas diversas atividades biológicas, sendo geralmente preparadas como infusão para o tratamento da febre, reumatismo, bronquite, doenças do estômago, e distúrbios digestivos, bem como hipertensão, febre amarela e gota (Alice *et al.*, 1991; Velazquez *et al.*, 2003; Bagetti *et al.*, 2011). Também podem reduzir o peso corporal e a pressão arterial, servir como um diurético, além de sua

comprovada atividade calmante e anti-inflamatória (Schmeda-Hirschmann *et al.*, 1987; Schapoval *et al.*, 1994). O extrato das folhas da pitangueira apresentou atividade citotóxica e anti-*Trypanosoma cruzi* com baixa toxicidade em estudos *in vitro* (Santos *et al.*, 2012).

A pitanga, ou seja, os frutos da pitangueira, também apresentam atividade antioxidante e atuam inibindo a peroxidação lipídica e na remoção de radicais livres (Velazquez *et al.*, 2003; Bagetti *et al.*, 2011; Celli *et al.*, 2011; Tambara *et al.*, 2018). Porém, ainda são poucos os trabalhos avaliando os efeitos dos extratos ou compostos isolados da pitanga, que apresentam diversos compostos com elevada capacidade antioxidante, sobre condições fisiológicas e patológicas. Portanto, este é um campo de pesquisa promissor que pode alavancar o consumo e a produção desta fruta nativa brasileira.

No estudo anterior foram separados e caracterizados de 27 compostos fenólicos da fruta pitanga roxa, sendo que a cianidina 3-*O*- glicosídio foi a principal antocianina encontrada na pitanga roxa, seguida da delfinidina 3-*O*- glicosídio, o que corresponde, respectivamente, a 82,7% e 16,1%, do conteúdo total de antocianina no extrato. Todos os compostos fenólicos não antocianínicos encontrados na pitanga roxa foram derivados de cinco agliconas: ácido quínico, ácido gálico, HHDP (hexahidroxidifenoil), miricetina e quercetina (Tambara *et al.*, 2018).

Devido ao seu sabor desejável e ao elevado conteúdo de compostos fenólicos (Rodriguez-Amaya *et al.*, 2008), a pitangueira é uma das árvores frutíferas mais promissoras para programas de exploração sustentável na Mata Atlântica Brasileira. No Brasil, as pitangueiras são cultivadas principalmente em hortas, pequenas propriedades agrícolas, ou de forma nativa. Atualmente, a Embrapa Clima Temperado localizada no município de Pelotas – RS possui um programa de melhoramento genético e expansão de produção desta árvore frutífera, o qual atua no fornecimento de mudas e programas informativos junto à comunidade. A região nordeste do Brasil é a única a explorar comercialmente esta fruta de alto potencial econômico. O valor comercial da pitanga resulta do seu elevado rendimento de polpa, valor nutritivo, sabor e aroma exóticos, atraindo, principalmente, os consumidores exigentes por produtos naturais e saudáveis (Donádio, 1983; Ferreira, 1987; Lederman *et al.*, 1992; Bezerra, 2000).

2.5 Obesidade

A obesidade é um sério problema de saúde pública que tem alcançado proporções alarmantes em todo o mundo e é um importante fator de risco para o desenvolvimento de diversas doenças de elevada morbidade e mortalidade como diabetes mellitus, dislipidemia, aterosclerose, hipertensão arterial, resistência à insulina, esteatose hepática, doença hepática não alcoólica entre outras (Cabrera e Jacob Filho, 2001; Sartorelli e Franco, 2003; França *et al.*, 2013).

A obesidade é uma doença metabólica crônica causada por um desequilíbrio entre ingestão e gasto energético, e é caracterizada pela deposição excessiva de gordura no tecido adiposo e outros órgãos internos como fígado, coração, músculo esquelético e pâncreas. O sobrepeso e a obesidade são definidos como um anormal ou excessivo acúmulo de gordura, o qual representa um risco para a saúde. A absorção de ácidos graxos e triglicerídeos e as respostas biológicas dependem da atividade lipolítica de enzimas presentes no metabolismo dos ácidos graxos do tecido adiposo (Adeneye *et al.*, 2010).

O tecido adiposo é um tecido dinâmico, multifuncional disperso por todo o organismo. Em mamíferos, existem dois tipos de tecido adiposo, o branco e o marrom. O branco é o predominante e serve como depósito lipídico, enquanto o marrom gera calor através do desacoplamento da cadeia respiratória mitocondrial. Os adipócitos armazenam energia na forma de triacilglicerídeos (TG), acumulando ou mobilizando TG em resposta aos requerimentos energéticos. Cerca de 30% das células no tecido adiposo são adipócitos maduros. Os outros dois terços são constituídos por pequenos vasos sanguíneos, tecido nervoso, fibroblastos e pré-adipócitos em diferentes estágios de diferenciação. Essa composição confere ao tecido adiposo uma grande plasticidade funcional, possibilitando sua fácil adaptação a grandes aumentos nos depósitos lipídicos, tanto por hipertrofia (aumento do tamanho de adipócitos já completamente diferenciados), como por hiperplasia (crescimento e diferenciação de células precursoras presentes no estroma) (Flynn e Woodhouse, 2008; Armani *et al.*, 2010).

Um considerável interesse tem sido despertado em todo o mundo no potencial de fitoquímicos para ajudar a tratar a obesidade. Os polifenóis constituem, dentre as classes de produtos naturais, os mais prováveis candidatos como agentes para prevenção contra obesidade. Diversos estudos sugerem que esses compostos podem modular o ciclo de vida dos adipócitos. A evidência mais forte para este efeito vem de derivados dos ácidos fenólicos (ácido clorogênico, por exemplo), flavonóis

(quercetina) e flavonas (luteolina). Essas classes de polifenóis são amplamente distribuídas nas plantas e, portanto, são consumidos regularmente como parte da dieta humana (Mezadri *et al.*, 2008; Jadeja *et al.*, 2011; Roh e Jung, 2012).

2.6 *Caenorhabditis elegans*

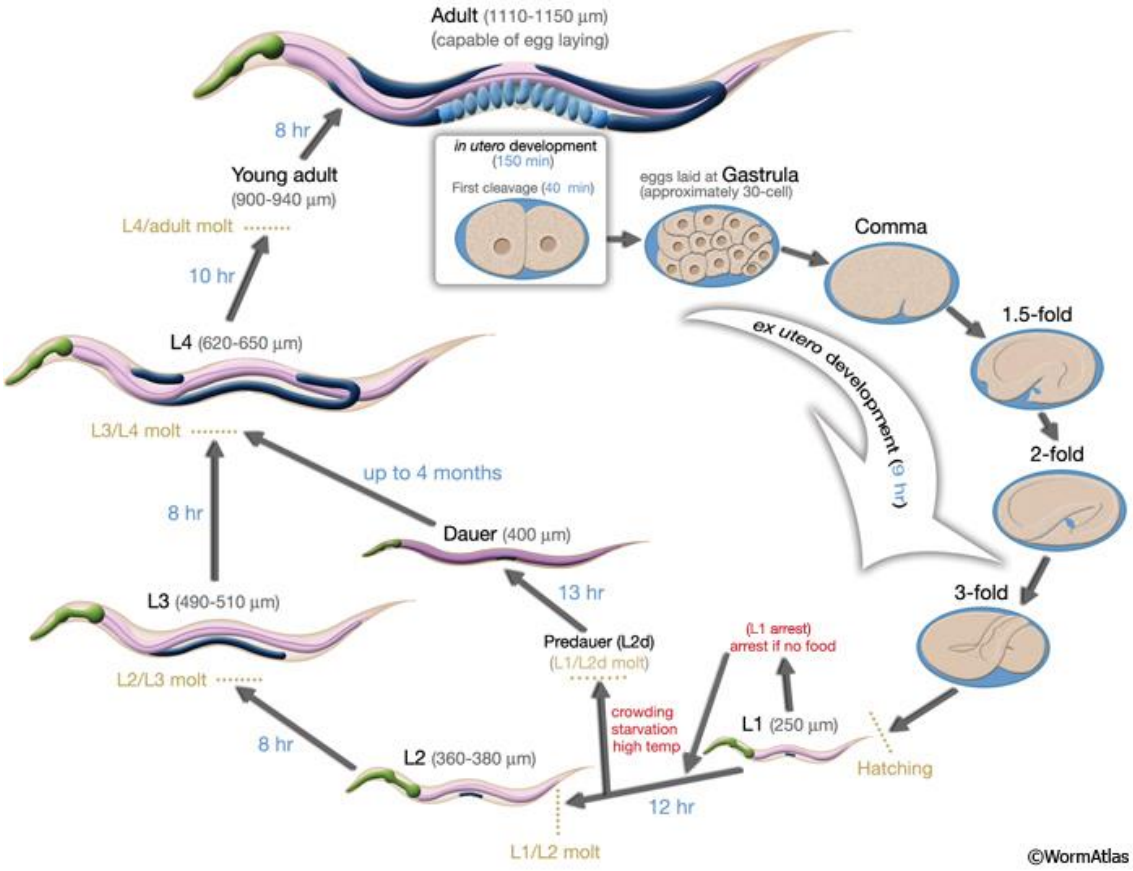
A avaliação da toxicidade de uma substância é realizada com o objetivo de predizer os efeitos nocivos que a mesma poderá desencadear quando da exposição humana pelas diversas vias. Para cumprir este propósito, o modelo animal é o mais utilizado nos estudos toxicológicos e requerido nos processos investigativos. Entretanto, a utilização de animais na pesquisa tem sido razão de diversas discussões em função do grande número necessário e do sofrimento causado, principalmente em relação aos estudos de toxicidade aguda. Existe uma tendência mundial para reavaliar a utilização de animais nos experimentos, concretizada a partir de um programa denominado de 3Rs que é assim denominado em função das iniciais, em inglês, de seus principais objetivos: 1) redução (*Reduction),* 2) refinamento *(Refinement)* e 3) substituição *(Replacement*), que de forma resumida significam a redução do número de animais utilizados na pesquisa, a melhora na condução dos estudos, no sentido de reduzir o sofrimento ao mínimo possível, e a busca de métodos alternativos que, por fim, substituam os testes *in vivo* (Cazarin *et al.*, 2004).

Diversas metodologias alternativas já foram implantadas, sendo este um processo complexo que abrange desde o seu desenvolvimento até sua aceitação e adoção por diversas organizações. Sendo assim, o presente trabalho apresenta os nematoides que são organismos pertencentes ao filo *Nemata* (*Nematoda*), e constituem o mais numeroso grupo de metazoários existente no solo (Politz e Philipp, 1992). O *Caenorhabditis elegans* pertence à família *Rhabditidae*, um diversificado grupo de nematoides com grande distribuição em habitat terrestres (Gilbert, 1988).

O *C. elegans* é um pequeno nematoide (cerca de 1 mm de comprimento) que tem sido utilizado como organismo modelo, por mais de 200 laboratórios mundiais. É um nematoide bacteriófago facilmente cultivado sob condições laboratoriais. Normalmente são mantidos à temperatura de 20ºC em placas de Petri com meio NGM (nematode growth media) e como fonte de alimento *Escherichia coli*, sendo possível obter-se um elevado número de organismos, o que é fundamental para um bom

modelo biológico (Gilbert, 1988). Os adultos são normalmente hermafroditas, sendo os machos raros (cerca de 0,2% da população total), cada hermafrodita produz uma descendência de cerca de 200-300 indivíduos (Bernt *et al.*, 1998). Após o desenvolvimento embrionário, o ovo eclode e liberta um jovem verme denominado larva L1, este se desenvolve por mais três sucessivas fases larvares (L2, L3 e L4) até chegar à fase adulta como representado na Figura 6 (Wood, 1988).

Figura 6: Ciclo de vida do *C. elegans* a 22°C



Fonte: WormAtlas

Este organismo possui uma série de características que o torna ideal para diversos tipos de estudos: possui um ciclo de vida curto, dimensões reduzidas, corpo transparente, um pequeno genoma e é fácil de cultivar (Diogo e Mota, 2001), sendo que o seu genoma foi totalmente sequenciado em 1998 (Park, 2013). Este nematoide foi proposto como um organismo modelo por Sydney Brenner em 1965 (Garcia-Sancho, 2012). Desde então, tem sido utilizado em estudos biológicos, genéticos e neurobiológicos de células eucariotas superiores.

Diversos estudos utilizando a suplementação com antioxidantes presentes naturalmente em alimentos ou isolados têm demonstrado resultados positivos em diversos modelos animais experimentais como o *C. elegans*. Entre eles podemos citar o extrato de café verde, o qual aumenta a resistência ao estresse oxidativo e atrasa o envelhecimento em *C. elegans* (Amigoni *et al.*, 2017), o extrato de maçã, que aumenta a vida útil, saúde e resistência ao estresse em *C. elegans (Vayndorf et al., 2013)* e um extrato rico em antocianinas do açaí que aumenta a resistência ao estresse e retarda os marcadores relacionados ao envelhecimento em *C. elegans* (Peixoto *et al.*, 2016). Ainda, estudos anteriores utilizando o extrato de pitanga roxa observaram que a fruta pitanga roxa (*Eugenia uniflora* L.) protege contra o estresse oxidativo e aumenta a expectativa de vida em *Caenorhabditis elegans* (Tambara *et al.*, 2018).

3 OBJETIVOS

3.1 Objetivo geral

Avaliar o potencial antioxidante do extrato de butiá e o potencial antiobesidade do extrato da pitanga roxa utilizando o modelo experimental *Caenorhabditis elegans*.

3.2 Objetivos específicos

- Determinar os efeitos do extrato etanólico da fruta butiá sobre a sobrevivência, longevidade e reprodução em *C. elegans*.
- Investigar o efeito do extrato de butiá sobre resistência ao estresse oxidativo induzido por paraquat e peróxido de hidrogênio em *C. elegans*.
- Padronizar um modelo de indução da obesidade pela dieta utilizando lipossomas com colesterol em *C. elegans.*
- Investigar o potencial do extrato etanólico da fruta pitanga roxa na redução de lipídios no modelo *C. elegans.*
- Analisar o efeito do extrato de pitanga roxa nas principais vias relacionadas ao metabolismo de lipídios em *C. elegans* utilizando cepas mutantes.

4 RESULTADOS

Os resultados que fazem parte desta tese estão apresentados sob as formas de Artigo científico e Manuscritos 1 e 2. As seções Materiais e Métodos, Resultados, Discussão, conclusão e Referências Bibliográficas encontram-se nos Artigo científico e Manuscritos.

4.1 Artigo científico

O seguinte artigo foi publicado em Janeiro de 2020 na revista Journal of Food Biochemistry.

Received: 23 July 2019 | Revised: 8 November 2019 | Accepted: 28 November 2019

DOI: 10.1111/jfbc.13139

FULL ARTICLE

Journal of Food Biochemistry WILEY

Butiá fruit extract (*Butia eriospatha*) protects against oxidative damage and increases lifespan on *Caenorhabditis elegans*

Andréia Limana Tambara | Élen Cristiane da Silveira | Ana Thalita Gonçalves Soares | Willian Goulart Salgueiro | Cristiane de Freitas Rodrigues | Jean Ramos Boldori | Daiana Silva de Ávila | Cristiane Casagrande Denardin

Biochemistry post-graduation program, Universidade Federal do Pampa, Uruguaiana, Brazil

Correspondence
Cristiane Casagrande Denardin, Biochemistry post-graduation program, Universidade Federal do Pampa, Campus Uruguaiana, BR 472, Km 592, Caixa Postal 118, Uruguaiana, RS, CEP: 97500-970, Brazil.
Email: cristianedenardin@unipampa.edu.br

Funding information
Empresa Brasileira de Pesquisa Agropecuária, Grant/Award Number: collaboration and supply of fruit samples; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: Financial support; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: Financial support

Abstract

Butiá (*Butia eriospatha*) is a fruit of a palm tree belonging to the family *Arecaceae*, native to South America. The aim of this study was to evaluate the antioxidant potential of butiá extract using *Caenorhabditis elegans* as animal model. Initially, we performed survival experiments, reproduction, resistance to oxidative stress (post or pre-treatment with paraquat or hydrogen peroxide), longevity, superoxide dismutase, and catalase GFP reporters' expression. We observed that butiá extract did not affect the worms' survival. Similarly, egg laying also showed no significant difference between treatments. None of the extract concentrations tested was able to significantly protect or reverse paraquat-induced oxidative stress. However, they were able to reverse the oxidative damage induced by hydrogen peroxide. In addition, butiá extract increased *C. elegans* lifespan under stress and not per se. Our results demonstrate that the Butiá is able to extend the lifespan of the nematode *C. elegans* and that this effect may be mediated by an induced resistance to oxidative stress.

Practical applications

The practical applications of this research are to expand and bring scientific knowledge to the population about the benefits of the consumption of this native fruit from the southern region of Brazil. Many fruits and other plant foods are consumed and spread with benefits without proper scientific proof of these benefits. This fruit is widely cultivated and its production and consumption can be expanded from these results. Still, we point out that this is the first time that the benefits of this fruit are studied.

KEYWORDS
antioxidant, *Butia eriospatha*, *C. elegans*, oxidative stress

1 | INTRODUCTION

Butiá (*Butia eriospatha*) is a fruit of a palm tree belonging to the family Arecaceae, native from South America. It has an edible fruit whose seeds are used to extract oil. In Brazil, it occurs endemically and naturally at open areas and forests with Araucaria trees in southern states of Parana, Santa Catarina, and Rio Grande do Sul. The ripe fruit can be eaten raw or used in the preparation of juices, wines, and liquors (Henderson, Galeano, & Bernal, 1995). Recent studies have demonstrated its richness in the antioxidant phytochemicals compounds, such as phenolic compounds (359.50 mg GAE 100 g^{-1}), carotenoids (3.85 ± 0.74 μg β-carotene g^{-1}), ascorbic acid (vitamin C) (9.351 ± 0.06 mg 100 g^{-1}), in vitro antioxidant capacity, reducing the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical

(253.80 ± 25.4 IC50 mg L^{-1}), and FRAP (ferric reducing antioxidant power) (9.32 ± 0.9 μmol $FeSO_4 \cdot 7H_2O$ g^{-1}fw), which analyzes the metal reduction capacity (Denardin et al., 2015). Although, it is a widely consumed fruit in southern Brazil, studies evaluating its antioxidant health benefits are still lacking in scientific literature.

Lately, a large amount of evidence has shown the key role of free radicals and other oxidants as responsible for aging and degenerative diseases associated with aging, such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction (Sousa et al., 2007). The production of free radicals is controlled in living organisms by various antioxidants mechanisms, which may have endogenous origin or are derived from the diet by consumption of fruits and vegetables (Halliwell & Gutteridge, 1998; Sousa et al., 2007).

Foods that provide essential nutrients, not only for life, but also bioactive compounds that promote the health benefits and reduce the risk of chronic diseases, are being discovered. This protective effect has been assigned to the biological properties such as antioxidant, anti-inflammatory, and hypocholesterolemic. Such effects may be promoted by nutrients such as vitamins C, A and E, and especially many bioactive compounds such as phenolic compounds, flavonoids, carotenoids, and other (Seifried, Anderson, Fisher, & Milner, 2007).

In recent times, the alternative/complimentary model *Caenorhabditis elegans* has become increasingly popular for the in vivo approach for the pharmacological studies of plant-derived bioactive compounds. *C. elegans* is a free-living nematode whose genome was completely sequenced in 1998. After incubation, they develop through four larval stages, from L1 to L4. Adult hermaphrodite worms can produce progeny of 200 on average during the reproduction period; *C. elegans* life cycle is relatively short, which makes this animal a good experimental organism for aging research (Park, 2013). Several studies provide evidence that this worm is responsive to various natural compounds, either by developing increased stress resistance and/or even lifespan extension (Saul, Pietsch, Menzel, & Steinberg, 2008).

Considering that butiá is a fruit heavily consumed in southern Brazil and has a great antioxidant capacity in vitro; this work is justified by the lack of research in vivo, once this fruit has many bioactive compounds that can act in a beneficial way to combat aging and its comorbidities. Therefore, it is important to expand the studies over the effects of natural antioxidants present in fruit extracts. Thus, this study aimed to evaluate the antioxidant effects of butiá extract in vivo in *C. elegans*.

2 | MATERIALS AND METHODS

2.1 | Preparation of butiá extract

Butiá fruits (*Butia eriospatha*) were collected in their mature stage at the EMBRAPA-CPACT-Pelotas/RS and frozen prior to transport. For the extraction of phenolic compounds, all the fruits were washed in the tap water and the core was removed, the sample consisting of fruit pulp and peel. For the extraction of phenolic compounds, one hundred grams of frozen samples were homogenized with 300 ml of an ethanol solution (95°GL) in a beaker protected from light using an ultra-turrax mixer for 5 min, and then were placed on a magnetic stirrer for 30 min. Samples were centrifuged for 5 min at 3,000 rpm and the supernatant was recovered. The residue was submitted to another extraction as described above, and the supernatant mixed with the above. The recovered supernatant was evaporated in a rotary apparatus using temperatures between 40 and 45°C and vacuum, and further lyophilized. The dried extract was resuspended in milli-q water.

The extract was used for the determination of total phenolics quantified by the Folin–Ciocalteu method (Swain & Hillis, 1959), and based on these results we choose the concentrations tested in this study. A standard curve of chlorogenic acid was used and we obtained a total phenolic value of 16,783.20 μg of chlorogenic acid equivalent/ml (CAE/ml). From that, we conducted all experiments at the following concentrations: 5, 50, 100, 250, and 500 μg of CAE/ml.

2.2 | Maintenance of *C. elegans*

C. elegans strains were kept in an incubator at 20°C in Petri dishes containing NGM (nematode growth media) and *Escherichia coli* OP50 as food source (Brenner, 1974). The strains used were N2 (wild type), CF1553 (muls84) [SOD-3::GFP], and GA800 (wuls151) [ctl-1 + ctl-2 + ctl-3:: GFP]. Worms were exposed to treatments at the first larval stage (L1). L1 worms were obtained by a synchronization process, which is based on the disruption of hermaphroditic pregnant worms using a lysing solution (1% NaOCl, 2.4% NaOH) in order to obtain the eggs. After 12–14 hr, the eggs hatch isolated, releasing L1 larva.

2.3 | Concentration-response curves and stress resistance assays

The L1 larvae (2000) obtained by the synchronization process were exposed to treatments with different concentrations of fruit extract for 30 min in a liquid medium containing 0.5% NaCl and in the absence of bacteria. At the end of treatment, worms were washed three times with 0.5% NaCl to remove the extract and then plated on NGM seeded with *E. coli* OP50. Survival parameter was evaluated by scoring the alive worms at the plates 24 hr after the end of the treatment.

The protection or reversal of induced oxidative damage was performed by using paraquat (1-1′-dimethyl-4-4′-bipyridinium dichloride) (0.5 mM) or hydrogen peroxide (H_2O_2) (0.6 mM) as prooxidant agents. The protective effect of the extract was evaluated by pre-exposure to extract (30 min) followed by postexposure (also 30 min) to the prooxidant agent. The reversal effect was assessed by pre-exposure to the stressor (30 min) followed by a postexposure to the extract (same 30 min). Three additional washes were done between the exposures in all assays. Survival was assessed after 24 hr by counting the surviving worms.

2.4 | Reproduction and lifespan assessments

The worms treated as described above were individually transferred to new media plates seeded with *E. coli* OP50 after 48 hr the end of exposure (L4 stage). Egg laying was measured by monitoring the number of eggs laid by worms every 24 hr during the breeding cycle.

For lifespan, after 48 hr of acute exposure to treatment, 20 worms from each concentration in each replicate were transferred individually to the new plates seeded with *E. coli* OP50. The worms were monitored and transferred to the new plates every day until the last worm died.

2.5 | Fluorescence-based assays

All fluorescence-based assays were conducted in a SpectraMax M5 microplate reader (Molecular Devices®).

2.5.1 | Quantification of SOD and CTL expression

Strains with the GFP expression (CF1553 [muls84] and GA800 [wuls154]) were exposed to treatment as described above and after washing transferred to 96-well plates with 200 µl of M9 buffer. The total fluorescence was measured at excitation: 485 nm and emission: 530 nm.

In addition, images from the fluorescent worms were acquired in the epifluorescence microscopy. For each slide, at least 30 treated worms were mounted on 2% agarose pads and anaesthetized with few drops of levamisole 22.5 µM. Fluorescence observations were performed under epifluorescence microscopy housed in air-conditioned room (20–22°C) for image acquisitions.

2.5.2 | ROS measurement

1500 wild-type L1 worms were preexposed to different concentrations of butiá extract, as previously described, and then were washed out from the media treatment to be exposed to 0.5 mM of H_2DCF-DA (2,7-dichlorofluorescein-diacetate) for 1 hr. After that, worms were one more time washed out from the media containing the fluorescent dye, to then be exposed to hydrogen peroxide (0.4 mM). ROS kinetic was observed within the worms by the fluorescence measurement (Exc: 485 nm; Em: 535 nm) for 1 hr, in the presence of hydrogen peroxide. The results are expressed as role-time curves.

2.6 | Statistics

Analyses were performed using GraphPad Prism version 5 (GraphPad Software) and SPSS version 16 for Windows. For longevity analyses we applied ANOVA for repeated measures and post hoc Tukey. The one-way ANOVA analysis was applied to all the other tests, followed by post hoc Tukey. All $p < .05$ values were considered statistically significant. The values expressed in percentage (%) were normalized taking a value of 100% for the control. In all figures, error bars represent the standard error of the mean.

3 | RESULTS

3.1 | Effect per se of butiá extract on *C. elegans*

We observed that none of the tested concentrations of butiá extract significantly altered worms' survival (F (5, 23) = 1.159, p = .3591) (Figure 1a), egg laying (F (5, 16) = 1.919, p = .1470) (Figure 1b), and lifespan (Figure 1c and Supplementary Table 1). These results suggest that the butiá extract has no toxic effects in the nematode *C. elegans*. In addition, we observed no toxicity in these parameters at concentrations up to 2,000 µg CAE/ml (data not shown).

3.2 | Butiá extract protect against oxidative stress induced by hydrogen peroxide

All tested concentrations of butiá extract were not able to significantly reverse the oxidative stress induced by hydrogen peroxide (F (6, 14) = 2.632, p = .0636) (Figure 2a). However, all concentrations were able to significantly protect against oxidative damage induced by hydrogen peroxide (F (6, 14) = 10.98, $p < .0001$) (Figure 2b). Regarding reproduction, hydrogen peroxide decreased egg laying of *C. elegans* and all the concentrations were able to reverse the reproductive damage, except the lowest one (5 µg CAE/ml) (F (6, 13) = 19.35, $p < .0014$) (Figure 2c). In postexposure to hydrogen peroxide (F (6, 12) = 0.7501, p = .6211) (Figure 2d) there were no significant differences in egg laying.

In longevity assays, we observed that the extract concentrations of 50, 250, and 500 µg CAE/ml were able to significantly increase the lifespan of worms, reversing the damage induced by hydrogen peroxide (Figure 2e), whereas only the highest concentration tested (500 µg CAE/ml) of the extract significantly increased the longevity of the nematodes (Figure 2f and Supplementary Table 2). In addition, we point out that butiá extract was not able to increase the longevity per se, but significantly increased the longevity against the hydrogen peroxide stressor.

When we measured the production of reactive species against the stressor hydrogen peroxide, all concentrations of butiá extract were able to reduce the production of ROS (F (6, 24) = 11.18, $p < .0001$) (Figure 2g).

3.3 | Oxidative stress induced by paraquat was partially reversed by butiá extract

In the experiments at which oxidative stress was induced by paraquat neither tested butiá extract concentrations were able to significantly

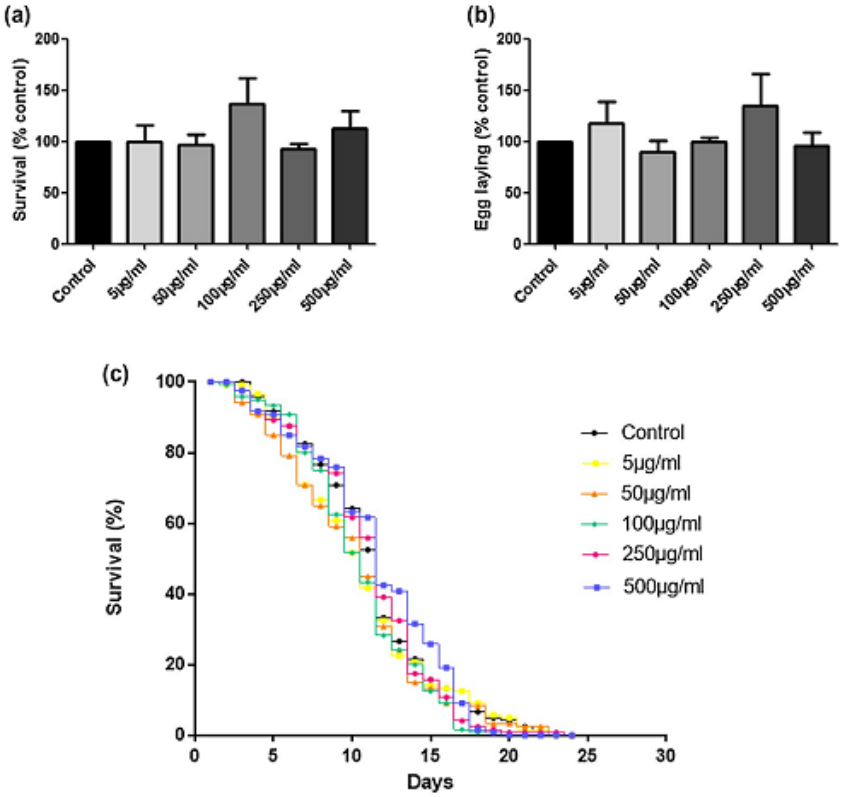


FIGURE 1 Butiá extract (*Butia eriospatha*) has no per se toxic effect in *C. elegans*. (a) Survival—live worms after 24 hr of treatment; (b) Reproduction—egg laying; (c) Lifespan. Values are mean ± standard error of three independent experiments. *$p < .05$ represent differences from the control

protect nor reverse the induced mortality (F (6, 21) = 8.841, $p < .0001$) (F (6, 14) = 7.126, $p < .0012$) (Figure 3a,b). When egg laying was assessed, no significant difference between the groups was noticed, thus paraquat did not affect *C. elegans* reproduction (F (6, 13) = 0.4888, $p = .8055$) (F (6, 13) = 2.249, $p = .1039$) (Figure 3c,d).

Intermediary extract concentrations (50, 100, and 250 µg CAE/ml) significantly increased the nematodes lifespan when administered as posttreatment against paraquat (Figure 3e and Supplementary Table 3) demonstrating a possible reversal effect exerted by the extract against the oxidizing agent. However, protection protocol was only effective in reversing the deleterious effect induced by paraquat over lifespan at the two highest extract concentrations (250 µg CAE/ml and 500 µg CAE/ml).

3.4 | Butiá extract per se did not alter the expression of GFP SOD and CTL reporters and catalase activity in *C. elegans*, but reverse the super expression of GFP SOD induced by H_2O_2

We evaluated the expression of SOD and CTL GFP reporters, and found that none butiá extract concentration was able to modify, per se, the expression of these endogenous antioxidant enzymes in *C. elegans* (Figure 4a,b). Similarly, the butiá extract per se was not able to modulate the CTL activity at any of the concentrations tested (Figure 4d). However, all the concentrations of butiá extract were able to reverse the super expression SOD GFP when exposed to H_2O_2 (Figure 4e). Thus, although butiá extract has shown protective and reversal effects against paraquat-induced damage over lifespan, reproduction, and survival tested stressors, this does not change the expression of SOD and CTL per se. Moreover, part of the results obtained in the protection and reversion of the oxidative damages observed in this work may be due to the modulation of superoxide dismutase enzyme expression by the butiá extract.

4 | DISCUSSION

In the present study we investigated the antioxidant potential of butiá extract using as experimental model the nematode *Caenorhabditis elegans*. In order to verify its antioxidant capacity, we performed survival, reproduction, lifespan, ROS measurement, and quantification of antioxidant enzymes in *C. elegans*.

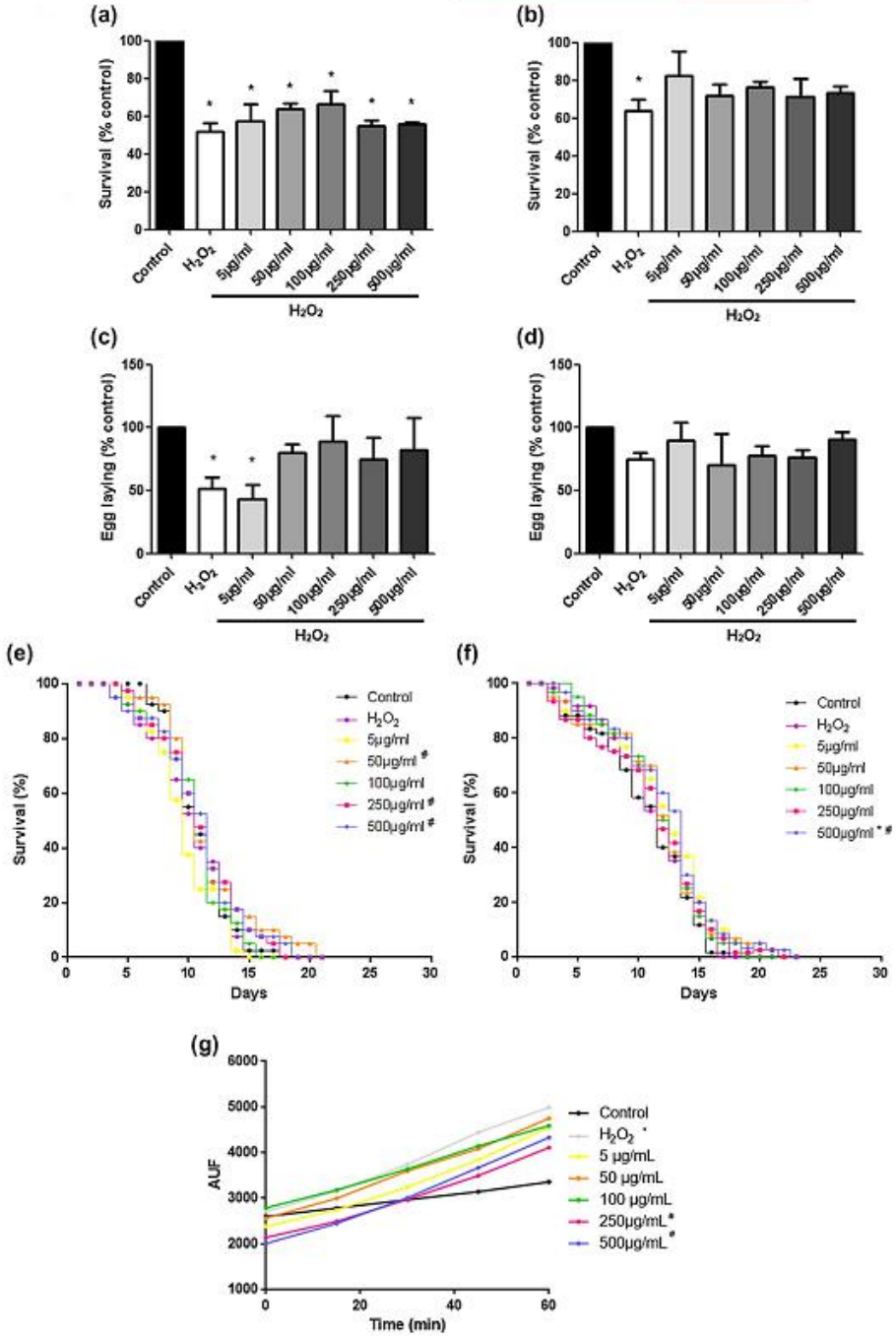


FIGURE 2 Butiá extract (*Butia eriospatha*) protects against oxidative damage induced by hydrogen peroxide (H_2O_2 0.6 mM) and significantly reduces the production of reactive species in C. *elegans*. Right column = protection test and left column = reversal test. (a) and (b): survival; (c) and (d): Reproduction—egg laying; (e) and (f): Lifespan. (g): DCF-DAD. Values are mean ± *SEM* of three independent experiments. $^*p < .05$ represent differences from the control. $^\#p < .05$ represent differences from H_2O_2 group

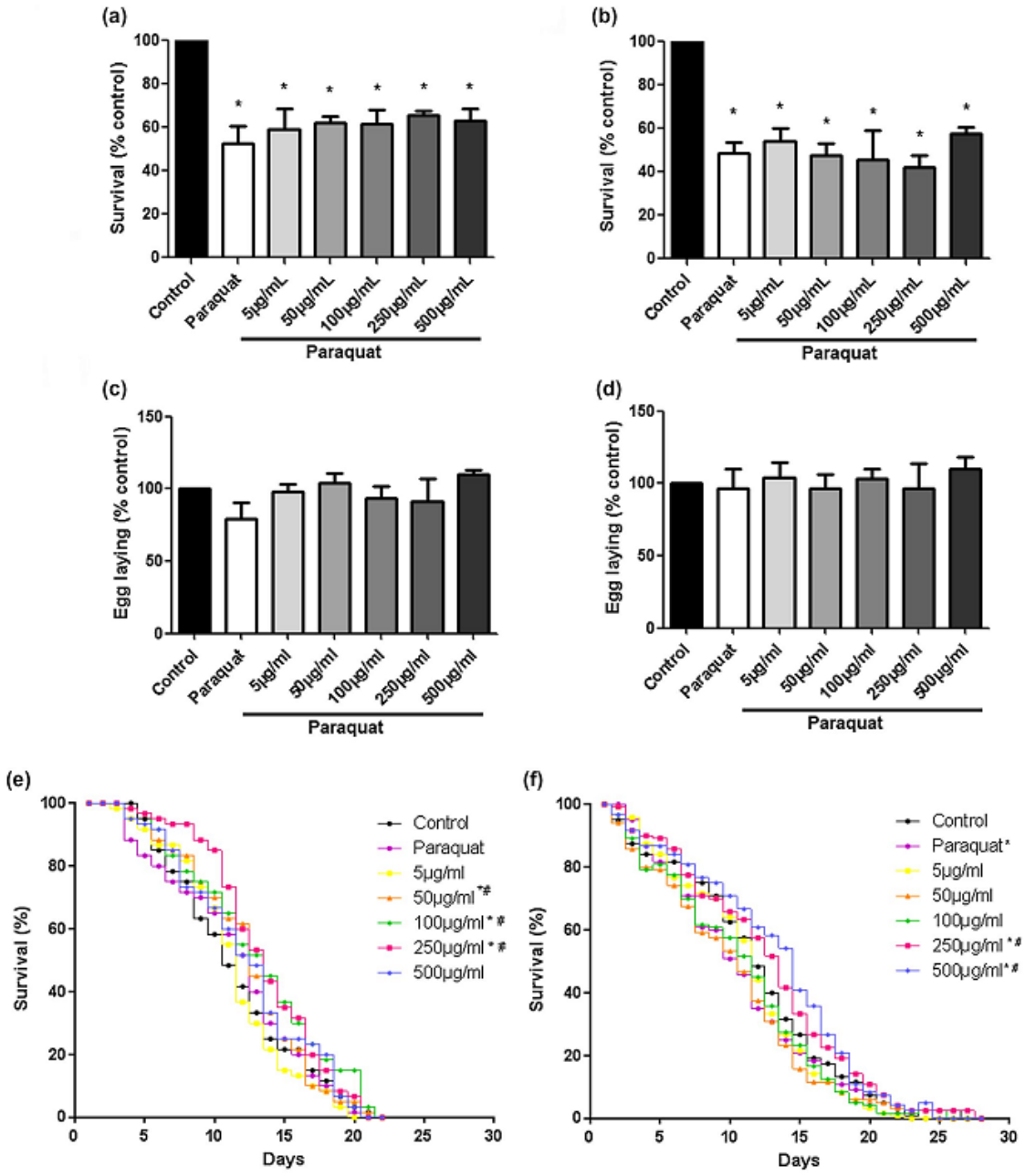


FIGURE 3 Butiá extract (*Butia eriospatha*) was able to protect and partially reverse the damage induced by Paraquat (0.5 mM) in *C. elegans*. Right column = protection test and left column = reversal test. (a) and (b): survival; (c) and (d): Reproduction—egg laying; (e) and (f): Lifespan. Values are mean ± *SEM* of three independent experiments. $^{*}p < .05$ represent differences from the control. $^{\#}p < .05$ represent differences from Paraquat group

According to Silveira and Collaborators (2005), species belonging to the *Palmae* family are very attractive from a chemical and pharmacological point of view, considering both plant and fruit. In recent years, a growing interest over unexplored native species, such as those from *Palmae* family, is due to the possible benefit that they may provide to human health. Such interest has been followed by extensive research over phytochemicals derived from the secondary plant metabolism, which are used as an indicative of these

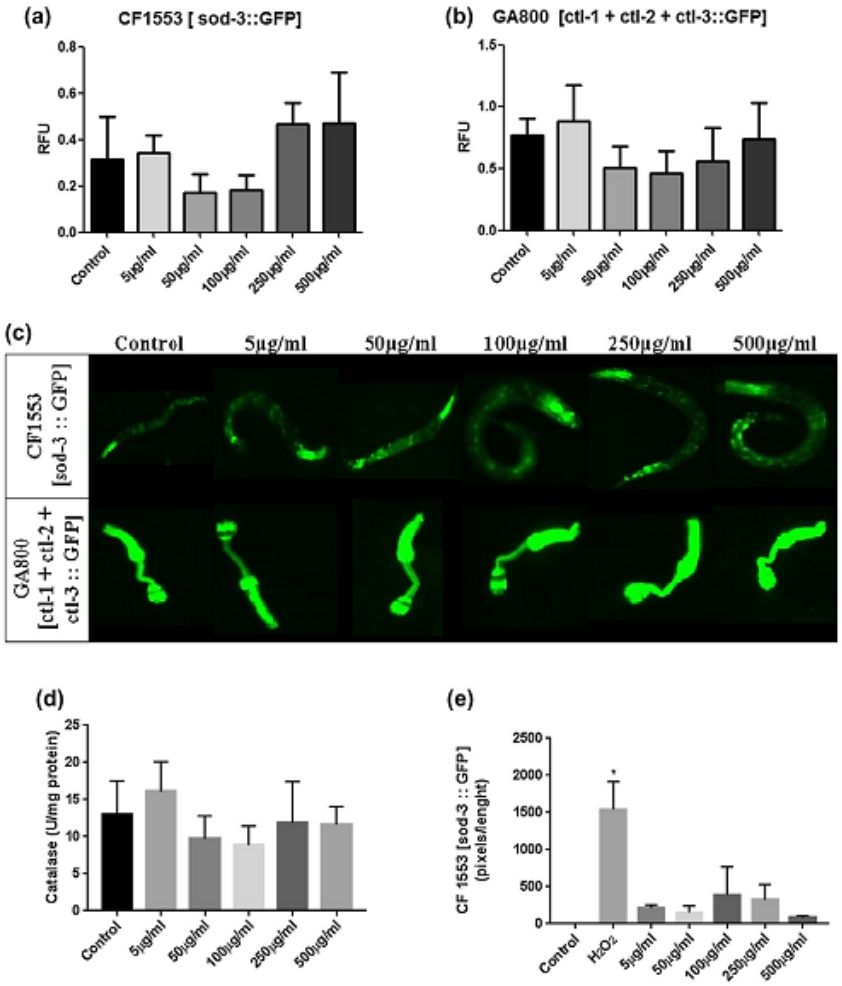


FIGURE 4 The expression of the enzyme superoxide dismutase (SOD) and catalase (CTL) did not altered by butiá extract (*Butia eriospatha*) per se in C. *elegans*. Butiá extract was able to reduce the super expression of the enzyme SOD induced by H_2O_2 (0.6 mM) and did not alter the activity of enzyme CTL per se. *$p < .05$ represent differences from the control

plants nutritional quality. Accordingly, antioxidant compounds can be extensively found among these aforementioned phytochemicals. Among the antioxidants present in most plant, the most active and frequently found are phenolic compounds such as flavonoids. The beneficial properties of these compounds can be attributed to its ability to directly and/or indirectly scavenge free radicals (Decker, 1997), as many of them have already been described for being capable of modulating intracellular cytoprotective machinery (Ghimire & Kim, 2017; Mitrasinovic, 2015).

Our results allowed us to observe that butiá extract, per se, did not show toxic effects on the nematode C. *elegans*, since it did not alter the nematodes' survival, reproduction, and lifespan (Figure 1). These results were expected, since it is a widely consumed fruit and it has no toxic effects described in the literature. Also, we extracted butiá's phenolic portion, mostly flavonoids, which are known for their lack of toxicity and potential pharmacological applications (Ma, Zeng, Sun, & Hu, 2014). Our rationale for choosing this specific butia's extract fraction was that in recent years, there has been increasing interest in identifying the molecules capable of slowing the deleterious effects of aging in vivo. Dietary phenolic compounds have emerged as promising candidates, mainly due to its antioxidant and anti-inflammatory properties, but also because a variety of them have shown effective bioavailability in humans (Manach, Williamson, Morand, Scalbert, & Remesy, 2005).

In order to verify whether the butiá extract indeed has antioxidant capacity in vivo we employed two pro-oxidizing agents: hydrogen peroxide (H_2O_2) (Figure 2) and paraquat (Figure 3). We observed that the two pro-oxidizing agents significantly induced mortality in C. *elegans*. We verified the putative stress-resistance effect provided by butiá extract by means of two different experimental designs: one

protocol aimed to evaluate the protection potential, by pretreating the worms with the extract; the second protocol aimed to evaluate the extract's potential damage reversing effect, following prooxidant exposure. None of the extract concentrations were able to reverse or protect the worms against the damage over survival induced by paraquat, usually a stronger stressor (Possik & Pause, 2015). Polyphenolic fraction of blueberry, another berry much similar to ours, also did not depict the ability to protect the nematodes from oxidative stress induced by paraquat, although conferred protection from thermic stress (Wilson et al., 2006). Moreover, all butiá extract concentrations reversed the damage over the nematodes' survival following hydrogen peroxide exposure, and the higher concentrations were also able to reverse the reproductive damage over egg laying (Figure 2). Considering that we use two stressors well known, some butiá extract concentrations significantly increased the lifespan of the nematodes. Notably, it has been shown that phenolic compounds have showed to be effective in modulating lifespan of *C. elegans*, especially quercetin, resveratrol, blueberry polyphenols, and catechin (Kampkotter et al., 2008; Saul, Pietsch, Menzel, Sturzenbaum, & Steinberg, 2009; Wilson et al., 2006; Wood et al., 2004).

The formation of free radicals in vivo occurs via catalytic action of enzymes during the electron transfer processes that occur in cellular metabolism and exposure to exogenous factors. However, free radicals intracellular concentration may increase due to their increased production or by deficiency of antioxidant mechanisms (Cerutti, 1991, 1994). The imbalance between the oxidants and antioxidants molecules has been called oxidative stress (Sies, 1993). Indeed, our results showed that an increased amount of ROS were being generated during H_2O_2 exposure, suggesting that the oxidative damage might be playing role in the observed deleterious effects following this oxidant exposure. However, higher concentrations of butiá extract were able to protect against ROS excessive production (Figure 2), which may explain their beneficial effects against induced oxidative damage over lifespan, acute stress resistance, and reproduction. Accordingly, many authors have demonstrated polyphenols capacity of neutralizing ROS in vivo by different mechanisms and in different organism models (Bonomo Lde et al., 2014; Jeong et al., 2014; Papandreou et al., 2012; Pavlica & Gebhardt, 2010). Such capacity was linked to many different physiological improvements over age-associated conditions and/or biochemical markers.

Aging occurs when the system experiences a gradual physiological and functional decline, resulting in poor maintenance of homeostasis, and, therefore, may lead to death (Harman, 1956). The free radicals theory of aging claims that the free radicals produced by aerobic metabolism may lead to the accumulation of oxidative damage, and thereby accelerate the aging process (Beckman & Ames, 1998). Flavonoids act as antioxidants in inactivation or blocking of free radicals, both in the cellular lipophilic and hydrophilic compartments. These compounds have the ability to donate hydrogen atoms and thus inhibit the chain reactions caused by free radicals. Flavonoids most investigated and presenting effective activity are: quercetin, myricetin, rutin, and naringenin (Arora, Nair, & Strasburg, 1998; Hartman & Shankel, 1990). Furthermore, flavonoids were already described for their capacity to modulate intracellular cytoprotective machinery (i.e., enzymatic induction, transcription factor activation), thus neutralizing the free radicals deleterious action by indirect means (Leonardo & Dore, 2011; Seibert, Maser, Schweda, Seibert, & Gulden, 2011).

Aerobic cells are protected from the action of superoxide and hydrogen peroxide by the action of superoxide dismutase, one metalloenzyme that converts superoxide radicals into hydrogen peroxide, and by the action of catalase, which converts hydrogen peroxide into water and molecular oxygen. Results of the measurement of SOD and CTL GFP reporters evidenced that there were no significant enzymatic modulation when worms were treated only with the butiá extract, which lead us to believe that the antioxidant effect observed is due to direct interaction between extracts compounds and ROS (Figure 4). Also, antioxidant enzymes induction is commonly suggested as a toxicity parameter, since their needing would indicate some sort of intracellular imbalance (Agus, Sumer, & Erkoc, 2015; Gemelli et al., 2011; Zhen et al., 2014). Then, lack of SOD and CTL induction may also be another indicative of butiá's extract low toxicity.

We did not discard the possibility of other enzymes regulation by butiá extract, such as glutathione and heat-shock proteins, both of which may be involved with the cytoprotection afforded by natural compounds (Bonomo Lde et al., 2014; Rezaizadehnajafi & Wink, 2014) Therefore, taking into account that the butiá extract has a rich composition of phenolic compounds, as well as a high concentration of carotenoids and vitamin C, we can infer that this fruit has great potential for use as an in vivo antioxidant due to its putative scavenger activity, since it did not show toxic effects, extended the nematode's lifespan, and was able to reverse the damage caused by the stressor hydrogen peroxide without modulating intracellular antioxidant machinery.

5 | CONCLUSION

Our results demonstrate, for the first time, that the Butiá is able to extend the lifespan of the nematode *C. elegans* and that this effect may be mediated by an induced resistance to oxidative stress. The Butiá extract was also able to protect and reverse the oxidative damage induced by hydrogen peroxide and the reactive oxygen species. Based on our results we conclude that Butiá extract per se did not show toxic effects in *C. elegans*, being healthier use and demonstrating that whole fruit extracts have optimal efficiency.

ACKNOWLEDGMENTS

The authors acknowledge to CAPES and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support; and Embrapa Clima Temperado for their collaboration and supply of fruit samples.

CONFLICTS OF INTEREST

All authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Tambara, L. A., performed the experiments with the worms and extract, and wrote the work.

Silveira, E. C., Boldori, J. R., and Rodrigues, C. F., performed the experiments with the worms and extract, and wrote the work.

Soares, A. T. G. and Salgueiro, W. G., performed the experiments with the fluorescent worms and extract.

Ávila, D. S., helped in writing work and reviewing.

Denardin, C. C., guided work and assisted in writing and execution.

All authors read and approved the final manuscript.

ORCID

Cristiane Casagrande Denardin https://orcid.org/0000-0002-0912-1357

REFERENCES

Agus, H. H., Sumer, S., & Erkoc, F. (2015). Toxicity and molecular effects of di-n-butyl phthalate (DBP) on CYP1A, SOD, and GPx in *Cyprinus carpio* (common carp). *Environmental Monitoring and Assessment*, *187*(7), 423. https://doi.org/10.1007/s10661-015-4622-3

Arora, A., Nair, M. G., & Strasburg, G. M. (1998). Structure-activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. *Free Radical Biology and Medicine*, *24*(9), 1355–1363. https://doi.org/10.1016/S0891-5849(97)00458-9

Beckman, K. B., & Ames, B. N. (1998). The free radical theory of aging matures. *Physiological Reviews*, *78*(2), 547–581. https://doi.org/10.1152/physrev.1998.78.2.547

Bonomo Lde, F., Silva, D. N., Boasquivis, P. F., Paiva, F. A., Guerra, J. F., Martins, T. A., … Oliveira Rde, P. (2014). Acai (*Euterpe oleracea* Mart.) modulates oxidative stress resistance in *Caenorhabditis elegans* by direct and indirect mechanisms. *PLoS ONE*, *9*(3), e89933. https://doi.org/10.1371/journal.pone.0089933

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics*, *77*(1), 71–94.

Cerutti, P. A. (1991). Oxidant stress and carcinogenesis. *European Journal of Clinical Investigation*, *21*(1), 1–5. https://doi.org/10.1111/j.1365-2362.1991.tb01350.x

Cerutti, P. A. (1994). Oxy-radicals and cancer. *Lancet*, *344*(8926), 862–863.

Decker, E. A. (1997). Phenolics: Prooxidants or antioxidants? *Nutrition Reviews*, *55*(11 Pt 1), 396–398. https://doi.org/10.1111/j.1753-4887.1997.tb01580.x

Denardin, C. C., Hirsch, G. E., da Rocha, R. F., Vizzotto, M., Henriques, A. T., Moreira, J. C. F., … Emanuelli, T. (2015). Antioxidant capacity and bioactive compounds of four Brazilian native fruits. *Journal of Food and Drug Analysis*, *23*(3), 387–398. https://doi.org/10.1016/j.jfda.2015.01.006

Gemelli, T., Carvalho, C. A. S., de Andrade, R. B., Guerra, R. B., Oliboni, L., Salvador, M., … Funchal, C. (2011). The organochalcogen 3-methyl-1-phenyl-2-(phenylseleno)oct-2-en-1-one induces oxidative stress in heart, liver, and kidney of rats. *Molecular and Cellular Biochemistry*, *355*(1–2), 167–172. https://doi.org/10.1007/s11010-011-0850-1

Ghimire, S., & Kim, M. S. (2017). Jujube (*Ziziphus Jujuba* Mill.) fruit feeding extends lifespan and increases tolerance to environmental stresses by regulating aging-associated gene expression in Drosophila. *Biogerontology*, *18*(2), 263–273. https://doi.org/10.1007/s10522-017-9686-8

Halliwell, B., & Gutteridge, J. M. C. (1998). *Free radicals in biology and medicine*. Oxford, UK: Oxford University Press.

Harman, D. (1956). Aging: A theory based on free radical and radiation chemistry. *The Journal of Gerontology*, *11*(3), 298–300. https://doi.org/10.1093/geronj/11.3.298

Hartman, P. E., & Shankel, D. M. (1990). Antimutagens and anticarcinogens: A survey of putative interceptor molecules. *Environmental and Molecular Mutagenesis*, *15*(3), 145–182. https://doi.org/10.1002/em.2850150305

Henderson, A., Galeano, G., & Bernal, R. (1995). *Field guide to the palms of the Americas*. Princeton, NJ: Princeton University Press.

Jeong, J. H., Kim, H. J., Park, S. K., Jin, D. E., Kwon, O. J., Kim, H. J., & Heo, H. J. (2014). An investigation into the ameliorating effect of black soybean extract on learning and memory impairment with assessment of neuroprotective effects. *BMC Complementary and Alternative Medicine*, *14*, 482. https://doi.org/10.1186/1472-6882-14-482

Kampkotter, A., Timpel, C., Zurawski, R. F., Ruhl, S., Chovolou, Y., Proksch, P., & Watjen, W. (2008). Increase of stress resistance and lifespan of *Caenorhabditis elegans* by quercetin. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *149*(2), 314–323. https://doi.org/10.1016/j.cbpb.2007.10.004

Leonardo, C. C., & Dore, S. (2011). Dietary flavonoids are neuroprotective through Nrf2-coordinated induction of endogenous cytoprotective proteins. *Nutritional Neuroscience*, *14*(5), 226–236. https://doi.org/10.1179/1476830511Y.0000000013

Ma, Y., Zeng, M., Sun, R., & Hu, M. (2014). Disposition of flavonoids impacts their efficacy and safety. *Current Drug Metabolism*, *15*(9), 841–864.

Manach, C., Williamson, G., Morand, C., Scalbert, A., & Remesy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition*, *81*(1 Suppl), 230S–242S.

Mitrasinovic, P. M. (2015). Sequence-dependent binding of flavonoids to duplex DNA. *Journal of Chemical Information and Modeling*, *55*(2), 421–433. https://doi.org/10.1021/ci5006965

Papandreou, M. A., Tsachaki, M., Efthimiopoulos, S., Klimis-Zacas, D., Margarity, M., & Lamari, F. N. (2012). Cell-line specific protection by berry polyphenols against hydrogen peroxide challenge and lack of effect on metabolism of amyloid precursor protein. *Phytotherapy Research*, *26*(7), 956–963. https://doi.org/10.1002/ptr.3670

Park, S.-K., & Park, S.-K. (2013). Electrolyzed-reduced water increases resistance to oxidative stress, fertility, and lifespan via insulin/IGF-1-like signal in *C. elegans*. *Biological Research*, *46*(2), 147–152. https://doi.org/10.4067/S0716-97602013000200005

Pavlica, S., & Gebhardt, R. (2010). Protective effects of flavonoids and two metabolites against oxidative stress in neuronal PC12 cells. *Life Sciences*, *86*(3–4), 79–86. https://doi.org/10.1016/j.lfs.2009.10.017

Possik, E., & Pause, A. (2015). Measuring oxidative stress resistance of *Caenorhabditis elegans* in 96-well microtiter plates. *Journal of Visualized Experiments*, *99*, e52746. https://doi.org/10.3791/52746

Rezaizadehnajafi, L., & Wink, M. (2014). EPs7630® from *Pelargonium sidoides* increases stress resistance in *Caenorhabditis elegans* probably via the DAF-16/FOXO pathway. *Phytomedicine*, *21*(4), 547–550. https://doi.org/10.1016/j.phymed.2013.10.027

Saul, N., Pietsch, K., Menzel, R., & Steinberg, C. E. (2008). Quercetin-mediated longevity in *Caenorhabditis elegans*: Is DAF-16 involved? *Mechanisms of Ageing and Development*, *129*(10), 611–613. https://doi.org/10.1016/j.mad.2008.07.001

Saul, N., Pietsch, K., Menzel, R., Sturzenbaum, S. R., & Steinberg, C. E. (2009). Catechin induced longevity in *C. elegans*: From key regulator genes to disposable soma. *Mechanisms of Ageing and Development*, *130*(8), 477–486. https://doi.org/10.1016/j.mad.2009.05.005

Seibert, H., Maser, E., Schweda, K., Seibert, S., & Gulden, M. (2011). Cytoprotective activity against peroxide-induced oxidative damage and cytotoxicity of flavonoids in C6 rat glioma cells. *Food and Chemical Toxicology*, *49*(9), 2398–2407. https://doi.org/10.1016/j.fct.2011.06.055

Seifried, H. E., Anderson, D. E., Fisher, E. I., & Milner, J. A. (2007). A review of the interaction among dietary antioxidants and reactive oxygen species. *Journal of Nutritional Biochemistry*, *18*(9), 567–579. https://doi.org/10.1016/j.jnutbio.2006.10.007

Sies, H. (1993). Strategies of antioxidant defense. *European Journal of Biochemistry*, *215*(2), 213–219. https://doi.org/10.1111/j.1432-1033.1993.tb18025.x

Silveira, C. S., Pessanha, C. M., Lourenço, M. C. S., Neves Junior, I., Menezes, F. S., & Kaplan, M. A. C. (2005). Atividade antimicrobiana dos frutos de *Syagrus oleracea* e *Mauritia vinifera*. *Revista Brasileira de Farmacognosia*, *15*(2), 143–148. https://doi.org/10.1590/S0102-695X2005000200013

Sousa, C. M. D. M., Silva, H. R. E., Vieira, G. M., Ayres, M. C. C., Da Costa, C. L. S., Araujo, D. S., … Chaves, M. H. (2007). Total phenolics and antioxidant activity of five medicinal plants. *Quimica Nova*, *30*(2), 351–355. https://doi.org/10.1590/S0100-40422007000200021

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture*, *10*, 63–68.

Wilson, M. A., Shukitt-Hale, B., Kalt, W., Ingram, D. K., Joseph, J. A., & Wolkow, C. A. (2006). Blueberry polyphenols increase lifespan and thermotolerance in *Caenorhabditis elegans*. *Aging Cell*, *5*(1), 59–68. https://doi.org/10.1111/j.1474-9726.2006.00192.x

Wood, J. G., Rogina, B., Lavu, S., Howitz, K., Helfand, S. L., Tatar, M., & Sinclair, D. (2004). Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature*, *430*(7000), 686–689. https://doi.org/10.1038/nature02789

Zhen, H., Wen, M., Yang, Y., Can, Z., Hui, G., Li, X., & Deli, L. (2014). Toxic effects of HgCl2 on activities of SOD, AchE and relative expression of SOD, AChE, CYP1A1 of zebrafish. *Ecotoxicology*, *23*(10), 1842–1845. https://doi.org/10.1007/s10646-014-1350-3

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Tambara AL, da Silveira ÉC, Soares ATG, et al. Butiá fruit extract (*Butia eriospatha*) protects against oxidative damage and increases lifespan on *Caenorhabditis elegans*. *J Food Biochem*. 2020;00:e13139. https://doi.org/10.1111/jfbc.13139

4.2 Manuscrito 1

O manuscrito está apresentado da mesma forma que foi submetido à revista Journal of Pharmacological and Toxicological Methods.

Development of novel obesity-inducing model by diet using liposomes with cholesterol in *Caenorhabditis elegans*

Andréia Limana Tambara [1], Cristiane Freitas Rodrigues [1], Luiz Torres Neto [1], Juliana Germano Ferst[1], Camila Cupper Vieira[2], Paulo Bayard Dias Gonçalves[1], Leticia Marques Colomé [1], Cristiane Casagrande Denardin[1*].

[1] Universidade Federal do Pampa (Unipampa), BR 472, Km 585, Caixa Postal 118, Uruguaiana, CEP 97501-970, RS, Brazil.

[2] Centro de Ciências Rurais, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.

*****Corresponding author.

Cristiane Casagrande Denardin. Address: Universidade Federal do Pampa – Campus Uruguaiana. BR 472 - Km 592 - Caixa Postal 118 - Uruguaiana – RS – Brazil – CEP: 97500-970. Phone: +55 55 3413 4321. E-mail: cristianedenardin@unipampa.edu.br

Abstract

Obesity is characterized by the abnormal or excessive fat accumulation in adipose tissue and represents one of the biggest global public health problems today. Several other common diseases such as diabetes and cardiovascular disease are associated with overweight and obesity. *Caenorhabditis elegans* has been widely used to evaluate the mechanisms of obesity, but there is still no model of oral ingestion that promotes an increase in the lipid content in the body of this nematode, mimicking the obesity. The purpose of this work was to develop a model of fat accumulation induction in the nematode *Caenorhabditis elegans* using liposomes with cholesterol as a model of hypercaloric diet. We observed that worms are able to feed liposomes similar to their ingestion of bacteria. An increase in the levels of triglycerides, cholesterol and glucose in worms was observed when feed with cholesterol-containing liposomes. From these results we concluded that liposomes containing cholesterol can be used to increase the levels of body fat and alter the signaling of lipid metabolism in *C. elegans*.

Keywords: *C. elegans*, liposomes, cholesterol, triglycerides, glucose.

1. Introduction

Obesity is probably one of the more serious metabolic disorders, and is being considered the most important nutritional disorder, being present in both developed and developing countries (Popkin & Doak, 1998). According to the World Health Organization (WHO), the occurrence of obesity in individuals reflects the interaction between dietary and environmental factors, in addition to genetic predisposition. Among the dietary factors, it is possible to highlight the excess of energy and, mainly, of lipids in the diet, favoring the increase of adiposity (Amine et al., 2003). Obesity is a significant risk factor for serious diseases, including type II diabetes, coronary heart disease, hypertension and certain forms of cancer (Barsh et al., 2000; Kopelman, 2000; Luchsinger, 2006).

Caenorhabditis elegans is a biological model that has several characteristics that make it ideal for obesity research. It is an easy-to-grow nematode, having a short life cycle, transparent body (Eisenmann, 2005), fully sequenced genome (Park & Park, 2013) in addition to high conservation of molecular and cellular pathways in relation to mammals. This variety of genetic and behavioral tools makes *C. elegans* an excellent system for unraveling complex molecular mechanisms. Thus, in recent years, the study of lipid metabolism in *C. elegans* has emerged as a new field for research, raising ideas about the regulation of the energy balance at the level of the whole organism (Ashrafi, 2007; Jones & Ashrafi, 2009; Yue et al., 2021).

C. elegans has several strains genetically modified for the study of obesity with genetic modifications in key genes linked to lipid metabolism that show lean or obese phenotypes. However, there is no well-defined protocol using oral obesity induction. Most studies using this nematode for obesity studies take into account the reduction of body lipid content from control worms (N2 strain) (Haerkens et al., 2022; Liu et al., 2018; Wang et al., 2022). Other studies add cholesterol, glucose or fatty acids to the

worm culture medium (NGM medium) along with the bacteria (Crawford et al., 2021; Deline et al., 2013; Kim et al., 2010; Lu & Qiu, 2017). However, these methods do not ensure that the worms are effectively consuming the added compounds. Some problems in the reproduction of this models are observed as: excessive growth of bacteria due to excess of nutrients in the plates; metabolism of the compounds added in the medium by the bacteria; and diffusion of the compounds in the medium, hindering ingestion by *C. elegans*. In the search for solutions to most of the problems mentioned above, there are studies evaluating the use of carriers such as liposomes and lipid vesicles for the delivery of substance orally in the *C. elegans* model (Perni et al., 2017; Shibamura et al., 2009).

C. elegans is an organism that does not produce cholesterol endogenously, requiring supplementation through the diet. For this reason, we chose to induce obesity through the diet, providing cholesterol-containing liposomes to *C. elegans*. Liposomes are colloidal structures similar in size to the microorganism *E. coli*, the main source of energy and nutrients in the diet offered to *C. elegans* in laboratory cultures. *C. elegans* are cholesterol auxotrophic and require a food supply of this sterol, therefore, cholesterol is one of the main sources of lipid in the diet, along with other fatty acids that are provided by bacteria (Kurzchalia & Ward, 2003; Watts & Ristow, 2017; Yue et al., 2021).

Structurally, liposomes are nanometric-sized vesicles composed of one or more concentric lipid bilayers, separated by an aqueous medium. They can encapsulate hydrophilic and/or lipophilic substances, such as cholesterol, which is one of the main source of lipid for *C. elegans*. Since they are biodegradable, biocompatible and non-immunogenic, liposomes are highly versatile for research, therapy and analytical

applications (Batista et al., 2007; F Puisieux, P Couvreur, J Delattre, 1996; R.R.C.New, 1990).

Although there are different animal models of mammals using different types of diet to induce obesity, or using genetic mutations to generate genetically modified animals, we must take into account the high cost and complexity of using these animal models for research. Therefore, the propose of a new obesity-inducing model by diet using an experimental model such as *C. elegans*, which is much cheaper and easy to manipulate, would be of paramount importance for basic research of molecules with potential effect on obesity before the use of more complex models such as mammals. Still, if we consider the great genetic homology between *C. elegans* and mammals Thus, the aim of this study is to propose a new model of obesity induction through the diet using cholesterol-containing liposomes in the *C. elegans* model.

2. Materials and methods

2.1. Materials

Lipoïd® S75 (soybean lecithin at 75% of phosphatidylcholine) was purchased from Lipoïd GmbH (Ludwigshafen, Germany). Cholesterol, fluorescein isothiocyanate-dextran (FD4) were purchased from Sigma-Aldrich. Water was purified on a Milli-Q system obtained from a Millipore® synergy system (Millipore, Billerica, Massachusetts, USA). All solvents were of analytical grade and used as such.

2.2. Liposome preparation by ethanol injection method

Liposomes were prepared by a ethanol injection method (Jaafar-Maalej et al., 2010). The phospholipids (80 mg) and cholesterol (40 mg) were dissolved in ethanol.

The resulting organic phase was injected from what source a syringe pump in distilled water (10 mL) under magnetic stirring. Spontaneous liposome formation occurred instantaneously as soon as the ethanolic solution was in contact with the distilled water. The liposome suspension was kept under stirring for 10 minutes at room temperature. Thereafter, the ethanol was removed by rotary evaporation (Rotavapor R-144; Buchi) under reduced pressure. Final cholesterol concentration in the formulation was 4 mg/mL.

Fluorescein-loaded liposomes were prepared as described above. The fluorescein (40 mg) was added to the organic phase before the injection into aqueous phase.

2.3. Liposomes Characterization

Mean particle size and polydispersity of liposomes were determined by photon correlation spectroscopy (PCS), using ZetaPlus (Brookhaven Instruments 27 Company, Holtsville, USA), after sample dilution in water. Zeta potential was measured by the electrophoretic mobility of liposomes in the same instrument. The pH was measured directly in the samples using the Denver Instrument VB-10 potentiometer, previously calibrated with buffer solutions for pH 4 and 7 calibration. All measures were performed in triplicate at 25°C.

2.4. Maintenance of *C. elegans*

C. elegans strains were kept in an incubator at 20°C in Petri dishes containing NGM (nematode growth media) and *Escherichia coli* OP50 as food source (Brenner, 1974). The strains used were N2 (wild type). Worms were exposed to treatments at the first larval stage (L1). L1 worms were obtained by a synchronization process, which is

based on the disruption of hermaphroditic pregnant worms using a lysing solution (1% NaOCl, 2.4% NaOH) in order to obtain the eggs. After 12-14 hours, the eggs hatch isolated, releasing L1 larva.

2.5. Liposomes with fluorescein

The N2 worms were exposed to liposomes containing fluorescein for 3 hours and afterwards they were washed with M9 solution and the images of the worms were captured using a fluorescence microscope (EVOS FLoid®) (Shibamura et al., 2009).

2.6. Survival

2000 N2 (wild type) were exposed in the first larval stage to treatment with cholesterol liposomes (50, 100 and 200 µl) for 48 hours on plates containing NGM and dead *Escherichia coli* OP50. After the survival test was performed by counting the live worms.

2.7. Reproduction

2000 N2 (wild type) were exposed in the first larval stage to treatment with cholesterol liposomes (50, 100 and 200 µl) for 48 hours on plates containing NGM and dead *Escherichia coli* OP50. Afterwards, the reproduction test was performed by counting the brood size during the entire reproductive cycle.

2.8. Lifespan

After 48 hours of chronic treatment, twenty worms in duplicate were transferred individually to new plates seeded with *E. coli* OP50 and liposomes (200 µl) of

cholesterol. The worms were monitored and transferred to new plates every day until the last worm died.

2.9. Body length and area

2000 N2 (wild type) were exposed in the first larval stage to treatment with cholesterol liposomes (200 µl) for 48 hours in plates containing NGM and dead *Escherichia coli* OP50. Afterwards, the images of the animals were made using a microscope. The length and area of the animals were traced from the tip of the head to the tip of the tail and were measured digitally using the ImageJ software.

2.10. Triglyceride, cholesterol, and glucose levels

The levels of triglycerides, cholesterol and glucose were quantified in the N2 worms treated as previously described. After 48 hours, in phase L4, the worms will be washed until all bacteria are removed. Then, the worms will be frozen twice, sonicated and centrifuged (1200 rpm 1.5 minutes). 50 µl of the supernatant will be transferred to 96-well microplates, which were quantified using the test kits (Labtest kit). For data normalization, protein will be measured in these samples using the Bradford colorimetric method (Bradford, 1976).

2.11. Determination of total lipid levels

To evaluate the total accumulation of lipids, 48h after the treatments, the worms were transferred to 96-well plates containing 200 µl of phosphate buffered saline and exposed to 5 µL of AdipoRed assay reagent (Nile red dye - Lonza). After 10 minutes of incubation, the animals' fluorescence was measured on a fluorimeter with excitation at 485 nm and emission at 572 nm. The images of the worms were captured using a

fluorescence microscope (EVOS FLoid®). Staining with Oil Red was also performed according to Escorcia et al. (2018) (Escorcia et al., 2018). Images of the worms were captured using an Olympus inverted microscope, 10X magnification.

2.12. Gene expression

Total RNA was extracted from nematode samples (3000 nematodes/sample) by using TRIzol® (Thermo Fisher Scientific, Middletown, VA), then RNA samples were reverse-transcribed by using high-capacity cDNA reverse transcription kit (Thermo Fisher Scientific, Inc.). Gene signal intensity was detected by StepOnePlus™ Real-Time PCR system (Applied Biosystems, Foster City, CA), then comparative threshold cycle method was used to express the results as fold change of gene expression.

2.13. Statistical analyses

Analyses were performed using GraphPad Prism version 6 (GraphPad Software). To analyze the results of longevity were applied ANOVA for repeated measures and post-hoc Tukey. The one-way ANOVA analysis was applied to all the other tests, followed by post-hoc Tukey. All $p < 0.05$ values were considered statistically significant. The values expressed in percentage (%) were normalized taking a value of 100% for the control. In all figures, error bars represent the standard error of the mean. All analyzes were performed in at least three independent experiments with replicates in duplicate.

3. Results

3.1. Analysis of liposomes

Liposomes presented standard characteristics. The values obtained by photon correlation spectroscopy analysis demonstrated that liposomes presented particle size in a nanometrical range (158.3±6.3 nm) (Ulrich, 2002), which can facilitate their ingestion by worms.

The polisipersion index, which is an indicative of the amplitude of the granulometric distribution, showed a narrow size distribution (0.11±0.01), since the lower the index values, the smaller the variability of size between the liposomes (Montefusco-Pereira et al., 2020). The liposomes presented pH value of 7.61±0.01 and zeta potential of – 39.67±7.08.

3.2. Oral administration of liposomes with fluorescence

We used liposomes loaded with the fluorescein reagent to evaluate the efficiency in the oral administration of substances in *C. elegans*. Ingestion of liposomes loaded with fluorescent dye resulted in successful oral delivery of products to the intestine of *C. elegans* (Figure 1A). We did not observe alterations in the pharyngeal pumping, indicating that the worms ingest the liposomes and the bacteria in the same way (Figure 1B).

3.3. Cholesterol liposomes did not cause toxic effects on *C. elegans*

In survival and reproduction tests, we observed that treatment with cholesterol-containing liposomes did not significantly change the survival and brood size of the worms (Figure 1C and 1D, respectively). We observed with these results that the cholesterol-containing liposomes did not cause toxicity in *C. elegans*.

3.4. Cholesterol liposomes increase triglyceride, cholesterol and glucose levels in *C. elegans*

When we analyzed the triglyceride levels in the worms, we found that treatment with cholesterol-containing liposomes significantly increased the levels of triglycerides in the volumes of 100 µL and 200 µL of liposomes (Figure 2A). Cholesterol and glucose levels increased significantly only in the volume of 200 µL of liposomes with cholesterol (Figure 2B and 2C, respectively). To assess the total accumulation of lipids, we chose only the volume of liposomes with the greatest significance (200 µL), which showed a high accumulation of lipids in the body of the worms. We observed that the worms treated with cholesterol-containing liposomes had a greater amount of lipids in the intestine region when we used the Nile red probe (Adipo Red Kit) and the Oil red stain (Figure 2D, 2E and 2F).

3.5. Cholesterol liposomes decrease *C. elegans* longevity, but do not affect their size

The exposure of worms to the volume of 200 µL of liposomes with cholesterol significantly decreased the life expectancy of *C. elegans* nematodes (Figure 3A). In addition, treatment with cholesterol-containing liposomes, despite significantly affecting the longevity of *C. elegans*, did not affect the size of the nematode in the length and body area assessments of the worm (Figure 3B and 3C, respectively). Therefore, these results demonstrate that cholesterol-containing liposomes did not affect the developmental stage of nematodes as expected.

3.6. The effects of cholesterol liposomes on transcription factors related to lipid metabolism

To explore the mechanisms of fat accumulation in *C. elegans* through the ingestion of cholesterol-containing liposomes we examined the expression level of mRNA genes related to lipid metabolism with real-time PCR analysis. Our results demonstrate that the significantly altered genes were fat-5 and fat-7 (Figure 3D), which are part of the lipogenesis pathway.

4. Discussion

Obesity arises when energy consumption, mainly stored as triglycerides, exceeds energy expenditure (Flier, 2004; Spiegelman & Flier, 2001). The etiology of obesity is complex and can be influenced by diet, developmental stage, age, physical activity and genes (Brockmann & Bevova, 2002; Jeffrey M Friedman, 2003). This condition has become a public health problem worldwide and there is clear evidence linking obesity with several pathologies, such as cardiovascular diseases, type II diabetes mellitus and dyslipidemia (Borges et al., 2017). Increasingly, factors related to obesity have been studied, and most studies use animal models of rodents, which have a high cost and ethical factors involved. In recent years there has been a significant increase in the number of studies using the *C. elegans* model evaluating the accumulation of triglycerides in these worms as an obesity-like phenotype, in addition to evaluating some metabolic pathways involved in the synthesis and degradation of triglycerides by genetic modifications (Kim et al., 2010; Liu et al., 2018; Wang et al., 2022; Yue et al., 2021).

While mammals have adipocytes as cells specialized in lipid storage and metabolism, *C. elegans* store fat in the form of droplets in intestinal cells and in hypodermic cells. Since *C. elegans* have transparent bodies, these fat deposits can be directly seen in intact animals (Kimura et al., 1997), or they may also be made of

biochemical fat composition determinations in *C. elegans* (Kurzchalia & Ward, 2003; Satouchi et al., 1993; Watts & Browse, 2002). This is one of the main determinants for nematodes to become a good model for obesity studies (Yue et al., 2021).

C. elegans uptakes chemicals via two major routes: ingestion and cuticle diffusion. Test chemicals can be either mixed with the nematode growth medium (NGM) plate, added onto the surface of the NGM plate, mixed with bacteria (*E. coli*) or added in the liquid medium. The use of live or dead *E. coli* should be considered when delivering compound given that live *E.coli* may metabolize target chemicals (Watts, 2009; Yue et al., 2019, 2021). As already reported, some of these delivery methods are being discussed due to difficulties in their reproducibility, due to the diffusion of the compounds in the NGM medium and metabolization by *E. coli*. Therefore, delivery methods that generate bacteria mimics were developed to increase drug uptake via ingestion, such as lipid vesicles and liposome-loaded (Perni et al., 2017; Shibamura et al., 2009).

Most studies evaluating the effect of different compounds on fat reduction in *C. elegans* use the N2 strain (control) or strains with modifications in target genes such as TUB-1, NHR-49, SBP-1, AAK-2, and CEBP-2 (Haerkens et al., 2022; Liu et al., 2018; Wang et al., 2022). Other studies promote the induction of lipid accumulation in the worms by adding high glucose or cholesterol to the NGM medium, demonstrating in their results an increase in the levels of triglycerides, glucose, cholesterol in the worm, and the visualization of lipid droplets throughout the worm's body (Kim et al., 2010; Lu & Qiu, 2017; Sulistiyani et al., 2017). Therefore, as it is already demonstrated in the literature that higher amounts of cholesterol promote an increase in the fat accumulation in *C. elegans* and that liposomes can be a good alternative to improve the delivery of compounds: the proposal of this work was to develop a new more

efficient method of delivery using cholesterol-containing liposomes to increase and improve uptake via ingestion and promote an obesity-like phenotype in worms.

As can be seen in our results, the cholesterol-containing liposomes had an average size of approximately 0.158 µm, that is, a smaller size than the *E. coli* bacteria (1 to 2 µm). However, we observed from the fluorescence images that the fluorescein-labeled liposomes were very well ingested, accumulating along the worms' intestine (Figure 1). A previous study by Shibamura and colleagues (Shibamura et al., 2009) proposed the use of liposome-loaded compounds as a method for oral administration of compounds and also observed that *C. elegans* similarly ingests liposomes in a wide range of size (0.1 to 5 µm). Liposomes are used as biocompatible carriers of drugs, peptides, proteins, plasma DNA, for pharmaceutical, cosmetic and biochemical purposes. The enormous versatility in particle size and physical parameters of lipids offers an attractive potential for building vehicles tailored to a wide range of applications (Roncato et al., 2019; Ulrich, 2002).

In addition, we observed that *C. elegans* did not show any difference in the consumption of the *E. coli* or liposomes, since we did not observe any difference in the pharyngeal pumping of the worms (Figure 1). Therefore, apparently, there is no distinction between the consumption of your usual food (bacteria) or the liposomes. Another issue that was observed is that cholesterol-containing liposomes do not promote significant effects on nematode survival and reproduction (Figure 1). Cholesterol is a structural component of animal membranes that influences fluidity, permeability, and formation of lipid microdomains. The nematode *C. elegans* is unable to synthesize cholesterol, so it requires a food supply of this sterol. Given the small amount of cholesterol needed for viability, it was assumed that cholesterol functions as a precursor to hormones derived from sterol, rather than playing a structural role in

the composition and fluidity of the membrane (Kurzchalia & Ward, 2003). *C. elegans* has no pathway for cholesterol synthesis, therefore, the increase in total cell lipid after incubation with cholesterol represents the efficiency in the absorption of cholesterol by nematodes (Sulistiyani et al., 2017).

The cholesterol-liposome-induced diet was able to significantly increase the levels of triglycerides, cholesterol, glucose, and total lipids in *C. elegans* (Figure 2A, 2B, 2C, 2D, 2E and 2F respectively). In addition, we observed a reduction in the life expectancy of nematodes, in the same way that occurs in already well-defined animal models of inducing obesity through the oral diet (Silva et al., 2020; Watts et al., 2003). Other studies using diets rich in glucose and cholesterol in *C. elegans* also observed an increase in the levels of triglycerides, cholesterol and lipid droplet markers such as Nile red and Oil red (Crawford et al., 2021; Kim et al., 2010; Lu & Qiu, 2017). Given the relevance of this issue, it is essential that scientists have reliable, faster, and lower cost alternative models for studying the mechanisms and effects of obesity primarily using an ingestion-induced model. These facts highlight the importance of this new model of induction of the obesity-like phenotype in *C. elegans* using oral administration of lipids such as cholesterol.

Energy balance is highly regulated and involves a complex interplay between food sensation, nutrient intake signals, transport and storage of nutrients, eating behavior, growth, reproduction and energy expenditure via basal metabolic rate and physical activity. It is likely that variants in genes encoding nutrient-sensing systems, metabolic enzymes, and central nervous system regulators are responsible, in part, for the weight gain, as human obesity is estimated to be 40-70% determined by genes (Flier, 2004; J M Friedman, 2000; Spiegelman & Flier, 2001; Watts, 2009).

Our results suggest that the fat accumulation effects of cholesterol-containing liposomes would be via lipogenesis, as the significantly altered genes were fat-5 and fat-7, as we can see in Figure 3. These are genes that play a crucial role in the regulation of lipogenesis targeting fatty acid biosynthesis (Yue et al., 2019). The fatty acid delta-9 desaturases are known targets for antiobesity agents due to the inhibition of fatty acid desaturation, which may lead to a reduced fat accumulation. In *C. elegans*, palmitoyl-CoA desaturase (fat-5) is responsible for the desaturation of palmitic acid (16:0) into palmitoleic acid (16:1), while the human stearoyl-CoA desaturase (SCD) homologs (fat-6 and fat-7) converts stearic acid (18:0) into oleic acid (18:1Δ9) (Farias-Pereira et al., 2020).

Changes in genes related to fat in *C. elegans*, cause imbalances in the composition of fatty acids and are associated with metabolic, physiological and behavioral factors. These include altered total fat levels, defects in sensory signaling, defects in neurotransmission and reduced adult life span (Brock et al., 2006; Kahn-Kirby et al., 2004; Lesa et al., 2003; Van Gilst et al., 2005). We believe that the alterations in the genes related to lipid metabolism pathway (fat-5 and fat-7) observed in this work, may be due to the production of signaling molecules from the high cholesterol ingested by *C. elegans*. However, we need further studies to elucidate this issue, since the function of cholesterol in *C. elegans* is not fully elucidated.

5. Conclusion

From these results we can conclude that the new obesity-inducing model using liposomes containing cholesterol (200µl) can be used to increase the levels of triglycerides, cholesterol and glucose, in addition to decreasing the life expectancy and modulated the lipid signaling of adult worms, similar to the diet-induced obesity in

animals. In addition, we did not observe toxic effects of liposomes with cholesterol in high concentrations in the nematode *C. elegans*. Therefore, this methodology can be used as a model of oral obesity induction in *C. elegans*. Our model used cholesterol as a source of lipids for supplementation in the diet of *C. elegans* since there are already studies in the literature demonstrating the increase in the body fat content of the worm with the increase of this sterol. However, we are carrying out more studies to assess whether the ingestion of liposomes with other fatty acids will also promote the same effects observed in this study.

6. Author contributions

Conceptualization, A.L.T.; methodology, A.L.T., C.F.R., L.T.N. and J.G.F; formal analysis, A.L.T., C.C.V. and C.F.R; investigation, A.L.T; writing—original draft preparation, A.L.T, L.M.C. and J.G.F.; writing—review and editing, L.M.C., P.B.D.G. and C.C.D.; supervision, C.C.D.; project administration, C.C.D.; funding acquisition, C.C.D., P.B.D.G. All authors have read and agreed to the published version of the manuscript.

7. Acknowledgements

This research was funded in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Unipampa for partial financial assistance to carry out this work.

8. Conflicts of Interest: The authors declare no conflict of interest.

9. References

Amine, E. K., Baba, N. H., Belhadj, M., Deurenberg-Yap, M., Djazayery, A., Forrestre, T., Galuska, D. A., Herman, S., James, W. P. T., M'Buyamba Kabangu, J. R., Katan, M. B., Key, T. J., Kumanyika, S., Mann, J., Moynihan, P. J., Musaiger, A. O., Olwit, G. W., Petkeviciene, J., Prentice, A., … Yach, D. (2003). Diet, nutrition and the prevention of chronic diseases. *World Health Organization - Technical Report Series*, *916*. https://doi.org/10.1093/ajcn/60.4.644a

Ashrafi, K. (2007). Obesity and the regulation of fat metabolism. In *WormBook : the online review of C. elegans biology*. https://doi.org/10.1895/wormbook.1.130.1

Barsh, G. S., Farooqi, I. S., & O'Rahilly, S. (2000). Genetics of body-weight regulation. *Nature*, *404*(6778), 644–651. https://doi.org/10.1038/35007519

Batista, C. M., Carvalho, C. M. B. de, & Magalhães, N. S. S. (2007). Lipossomas e suas aplicações terapêuticas: estado da arte. *Revista Brasileira de Ciências Farmacêuticas*, *43*(2), 167–179. https://doi.org/10.1590/S1516-93322007000200003

Borges, J. G., Sá, J. A. R. de, Bezerra, K. S. P., Rolin, L. A., & Almeida, J. R. G. da S. (2017). TECHNOLOGICAL MAPPING OF OBESITY TREATMENTS USING NATURAL COMPOUNDS OF FRUITS. *Revista GEINTEC*, *7*(1), 3646–3654. https://doi.org/10.7198/S2237-072220170001005

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254. https://doi.org/10.1006/abio.1976.9999

Brenner, S. (1974). The genetics of Caenorhabditis elegans. *Genetics*, *77*(1), 71–94.

Brock, T. J., Browse, J., & Watts, J. L. (2006). Genetic regulation of unsaturated fatty acid composition in C. elegans. *PLoS Genetics*, *2*(7), e108.

https://doi.org/10.1371/journal.pgen.0020108

Brockmann, G. A., & Bevova, M. R. (2002). Using mouse models to dissect the genetics of obesity. *Trends in Genetics : TIG*, *18*(7), 367–376. https://doi.org/10.1016/s0168-9525(02)02703-8

Crawford, N., Martell, M., Nielsen, T., Khalil, B., Imtiaz, F., Nguidjo, E., Newell-Caito, J. L., Bornhorst, J., Schwerdtle, T., & Caito, S. W. (2021). Methylmercury-Induced Metabolic Alterations in Caenorhabditis elegans Are Diet-Dependent. *Toxics*, *9*(11). https://doi.org/10.3390/toxics9110287

Deline, M. L., Vrablik, T. L., & Watts, J. L. (2013). Dietary supplementation of polyunsaturated fatty acids in Caenorhabditis elegans. *Journal of Visualized Experiments : JoVE*, *81*. https://doi.org/10.3791/50879

Eisenmann, D. M. (2005). WormBook. *WormBook : The Online Review of C. Elegans Biology*.

Escorcia, W., Ruter, D. L., Nhan, J., & Curran, S. P. (2018). Quantification of Lipid Abundance and Evaluation of Lipid Distribution in Caenorhabditis elegans by Nile Red and Oil Red O Staining. *Journal of Visualized Experiments : JoVE*, *133*. https://doi.org/10.3791/57352

F Puisieux, P Couvreur, J Delattre, J.-P. D. (1996). Liposomes, New Systems and New Trends in their Applications. In E. de Santé (Ed.), *Journal of Drug Targeting* (Vol. 4, Issue 2). Taylor & Francis. https://doi.org/10.3109/10611869609046271

Farias-Pereira, R., Zhang, Z., Park, C.-S., Kim, D., Kim, K.-H., & Park, Y. (2020). Butein inhibits lipogenesis in Caenorhabditis elegans. *BioFactors*, *46*(5), 777–787. https://doi.org/https://doi.org/10.1002/biof.1667

Flier, J. S. (2004). Obesity wars: molecular progress confronts an expanding epidemic. *Cell*, *116*(2), 337–350. https://doi.org/10.1016/s0092-8674(03)01081-x

Friedman, J M. (2000). Obesity in the new millennium. *Nature*, *404*(6778), 632–634. https://doi.org/10.1038/35007504

Friedman, Jeffrey M. (2003). A war on obesity, not the obese. *Science (New York, N.Y.)*, *299*(5608), 856–858. https://doi.org/10.1126/science.1079856

Haerkens, F., Kikken, C., Kirkels, L., van Amstel, M., Wouters, W., van Doornmalen, E., Francke, C., & Hughes, S. (2022). A new use for old drugs: identifying compounds with an anti-obesity effect using a high through-put semi-automated Caenorhabditis elegans screening platform. *Heliyon*, *8*(8), e10108. https://doi.org/https://doi.org/10.1016/j.heliyon.2022.e10108

Jaafar-Maalej, C., Diab, R., Andrieu, V., Elaissari, A., & Fessi, H. (2010). Ethanol injection method for hydrophilic and lipophilic drug-loaded liposome preparation. *Journal of Liposome Research*, *20*(3), 228–243. https://doi.org/10.3109/08982100903347923

Jones, K. T., & Ashrafi, K. (2009). Caenorhabditis elegans as an emerging model for studying the basic biology of obesity. *Disease Models & Mechanisms*, *2*(5–6), 224–229. https://doi.org/10.1242/dmm.001933

Kahn-Kirby, A. H., Dantzker, J. L. M., Apicella, A. J., Schafer, W. R., Browse, J., Bargmann, C. I., & Watts, J. L. (2004). Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling in vivo. *Cell*, *119*(6), 889–900. https://doi.org/10.1016/j.cell.2004.11.005

Kim, H. M., Do, C.-H., & Lee, D. H. (2010). Characterization of taurine as anti-obesity agent in C. elegans. *Journal of Biomedical Science*, *17 Suppl 1*(Suppl 1), S33. https://doi.org/10.1186/1423-0127-17-S1-S33

Kimura, K. D., Tissenbaum, H. A., Liu, Y., & Ruvkun, G. (1997). daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis

elegans. *Science (New York, N.Y.)*, *277*(5328), 942–946. https://doi.org/10.1126/science.277.5328.942

Kopelman, P. G. (2000). Obesity as a medical problem. *Nature*, *404*(6778), 635–643. https://doi.org/10.1038/35007508

Kurzchalia, T. V, & Ward, S. (2003). Why do worms need cholesterol? *Nature Cell Biology*, *5*(8), 684–688. https://doi.org/10.1038/ncb0803-684

Lesa, G. M., Palfreyman, M., Hall, D. H., Clandinin, M. T., Rudolph, C., Jorgensen, E. M., & Schiavo, G. (2003). Long chain polyunsaturated fatty acids are required for efficient neurotransmission in C. elegans. *Journal of Cell Science*, *116*(Pt 24), 4965–4975. https://doi.org/10.1242/jcs.00918

Liu, J., Peng, Y., Yue, Y., Shen, P., & Park, Y. (2018). Epigallocatechin-3-Gallate Reduces Fat Accumulation in Caenorhabditis elegans. *Preventive Nutrition and Food Science*, *23*(3), 214–219. https://doi.org/10.3746/pnf.2018.23.3.214

Lu, Z., & Qiu, Z. (2017). High glucose concentration restricts fat consumption in Caenorhabditis elegans. *International Journal of Clinical and Experimental Medicine*, *10*(7), 10554–10559.

Luchsinger, J. A. (2006). A Work in Progress: The Metabolic Syndrome. *Science of Aging Knowledge Environment*, *2006*(10), pe19–pe19. https://doi.org/10.1126/sageke.2006.10.pe19

Montefusco-Pereira, C. V, Formicola, B., Goes, A., Re, F., Marrano, C. A., Mantegazza, F., Carvalho-Wodarz, C., Fuhrmann, G., Caneva, E., Nicotra, F., Lehr, C.-M., & Russo, L. (2020). Coupling quaternary ammonium surfactants to the surface of liposomes improves both antibacterial efficacy and host cell biocompatibility. *European Journal of Pharmaceutics and Biopharmaceutics*, *149*, 12–20. https://doi.org/https://doi.org/10.1016/j.ejpb.2020.01.013

Park, S.-K., & Park, S.-K. (2013). Electrolyzed-reduced water increases resistance to oxidative stress, fertility, and lifespan via insulin/IGF-1-like signal in C. elegans. *Biological Research*, *46*(2), 147–152. https://doi.org/10.4067/S0716-97602013000200005

Perni, M., Aprile, F. A., Casford, S., Mannini, B., Sormanni, P., Dobson, C. M., & Vendruscolo, M. (2017). Delivery of Native Proteins into C. elegans Using a Transduction Protocol Based on Lipid Vesicles. *Scientific Reports*, *7*(1), 15045. https://doi.org/10.1038/s41598-017-13755-9

Popkin, B. M., & Doak, C. M. (1998). The Obesity Epidemic Is a Worldwide Phenomenon. *Nutrition Reviews*, *56*(4), 106–114. https://doi.org/10.1111/j.1753-4887.1998.tb01722.x

R.R.C.New. (1990). *Liposomes : a practical approach* (1997th ed.). Oxford : IRL Press.

Roncato, J. F. F., Camara, D., Brussulo Pereira, T. C., Quines, C. B., Colomé, L. M., Denardin, C., Haas, S., & Ávila, D. S. (2019). Lipid reducing potential of liposomes loaded with ethanolic extract of purple pitanga (Eugenia uniflora) administered to Caenorhabditis elegans. *Journal of Liposome Research*. https://doi.org/10.1080/08982104.2018.1552705

Satouchi, K., Hirano, K., Sakaguchi, M., Takehara, H., & Matsuura, F. (1993). Phospholipids from the free-living nematode Caenorhabditis elegans. *Lipids*, *28*(9), 837–840. https://doi.org/10.1007/BF02536239

Shibamura, A., Ikeda, T., & Nishikawa, Y. (2009). A method for oral administration of hydrophilic substances to Caenorhabditis elegans: Effects of oral supplementation with antioxidants on the nematode lifespan. *Mechanisms of Ageing and Development*, *130*(9), 652–655.

https://doi.org/https://doi.org/10.1016/j.mad.2009.06.008

Silva, T. M. da, Oliveira, F. M. de, Rodrigues, K. C. P., Nobre, L. R., & Brito, M. L. (2020). Uso De Modelos Animais Na Indução Da Obesidade E Alterações Fisiológicas / Use of Animal Models in Inducing Obesity and Physiological Changes. *Brazilian Journal of Development*, *6*(9), 66278–66286. https://doi.org/10.34117/bjdv6n9-165

Spiegelman, B. M., & Flier, J. S. (2001). Obesity and the regulation of energy balance. *Cell*, *104*(4), 531–543. https://doi.org/10.1016/s0092-8674(01)00240-9

Sulistiyani, Purwakusumah, E. P., & Andrianto, D. (2017). In Vivo Inhibition of Lipid Accumulation in*Caenorhabditis elegans*. *IOP Conference Series: Earth and Environmental Science*, *58*, 12067. https://doi.org/10.1088/1755-1315/58/1/012067

Ulrich, A. S. (2002). Biophysical aspects of using liposomes as delivery vehicles. *Bioscience Reports*, *22*(2), 129–150. https://doi.org/10.1023/a:1020178304031

Van Gilst, M. R., Hadjivassiliou, H., Jolly, A., & Yamamoto, K. R. (2005). Nuclear hormone receptor NHR-49 controls fat consumption and fatty acid composition in C. elegans. *PLoS Biology*, *3*(2), e53. https://doi.org/10.1371/journal.pbio.0030053

Wang, Y., Xiang, Y.-F., & Liu, A.-L. (2022). Comparative and Combined Effects of Epigallocatechin-3-gallate and Caffeine in Reducing Lipid Accumulation in Caenorhabditis elegans. *Plant Foods for Human Nutrition (Dordrecht, Netherlands)*, *77*(2), 279–285. https://doi.org/10.1007/s11130-022-00978-z

Watts, J. L. (2009). Fat synthesis and adiposity regulation in Caenorhabditis elegans. *Trends in Endocrinology and Metabolism: TEM*, *20*(2), 58–65. https://doi.org/10.1016/j.tem.2008.11.002

Watts, J. L., & Browse, J. (2002). Genetic dissection of polyunsaturated fatty acid synthesis in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(9), 5854–5859. https://doi.org/10.1073/pnas.092064799

Watts, J. L., Phillips, E., Griffing, K. R., & Browse, J. (2003). Deficiencies in C20 polyunsaturated fatty acids cause behavioral and developmental defects in Caenorhabditis elegans fat-3 mutants. *Genetics*, *163*(2), 581–589. https://doi.org/10.1093/genetics/163.2.581

Watts, J. L., & Ristow, M. (2017). Lipid and Carbohydrate Metabolism in Caenorhabditis elegans. *Genetics*, *207*(2), 413–446. https://doi.org/10.1534/genetics.117.300106

Yue, Y., Li, S., Shen, P., & Park, Y. (2021). Caenorhabditis elegans as a model for obesity research. *Current Research in Food Science*, *4*, 692–697. https://doi.org/https://doi.org/10.1016/j.crfs.2021.09.008

Yue, Y., Shen, P., Chang, A. L., Qi, W., Kim, K.-H., Kim, D., & Park, Y. (2019). trans-Trismethoxy resveratrol decreased fat accumulation dependent on fat-6 and fat-7 in Caenorhabditis elegans. *Food & Function*, *10*(8), 4966–4974. https://doi.org/10.1039/c9fo00778d

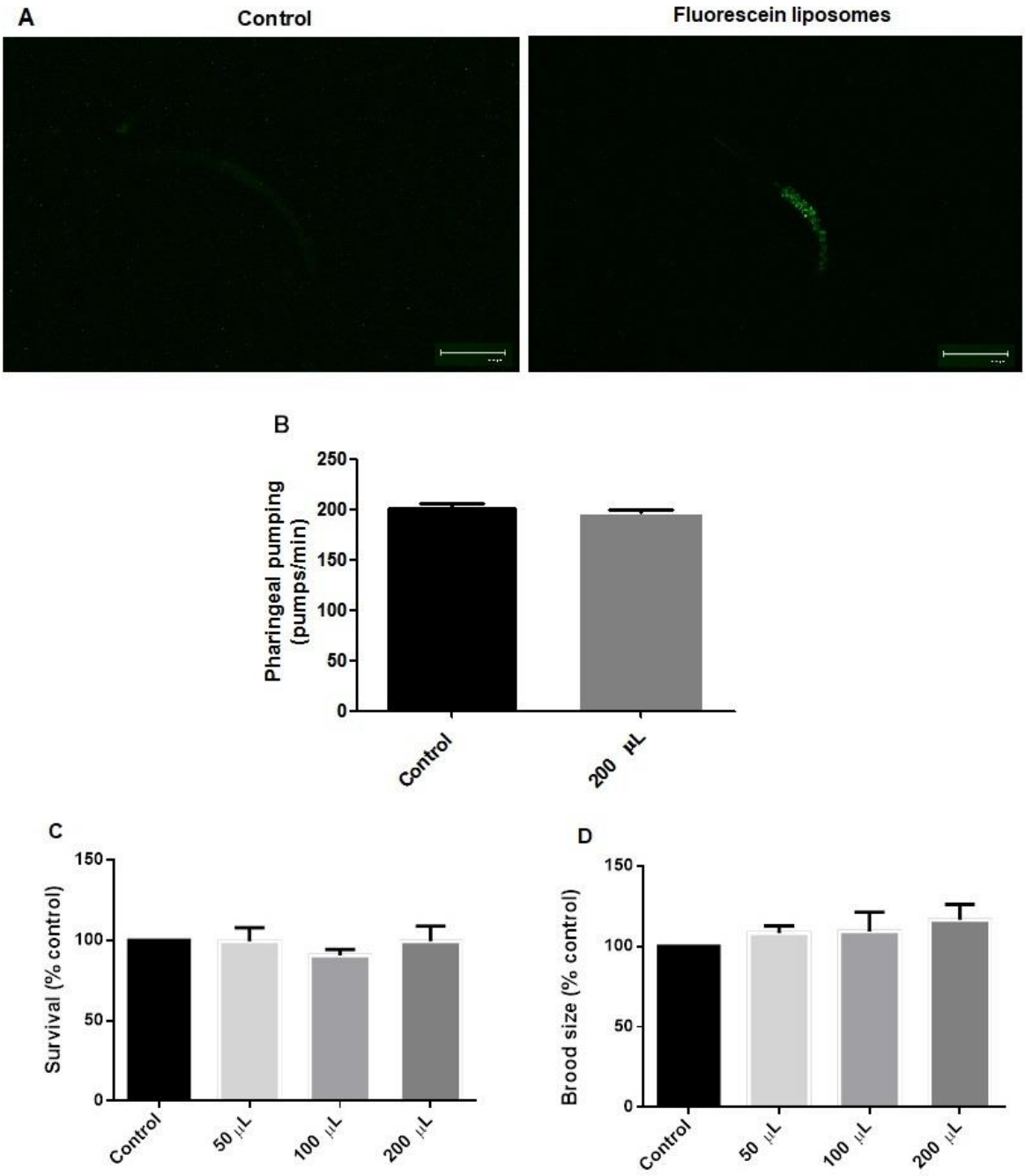


Figure 1. Oral administration of liposomes. (A). Worms (N2 strain) control and Worms (N2 strain) feed with fluorescein-liposomes. Representative photos (100 µm magnification). Effect of treatment of liposomes with cholesterol on (B) pharyngeal pumping (C) survival and (D) reproduction in *C. elegans*. Values are mean ± standard

error. *p<0.05 represent differences of control. All analyzes were performed in at least three independent experiments with replicates in duplicate.

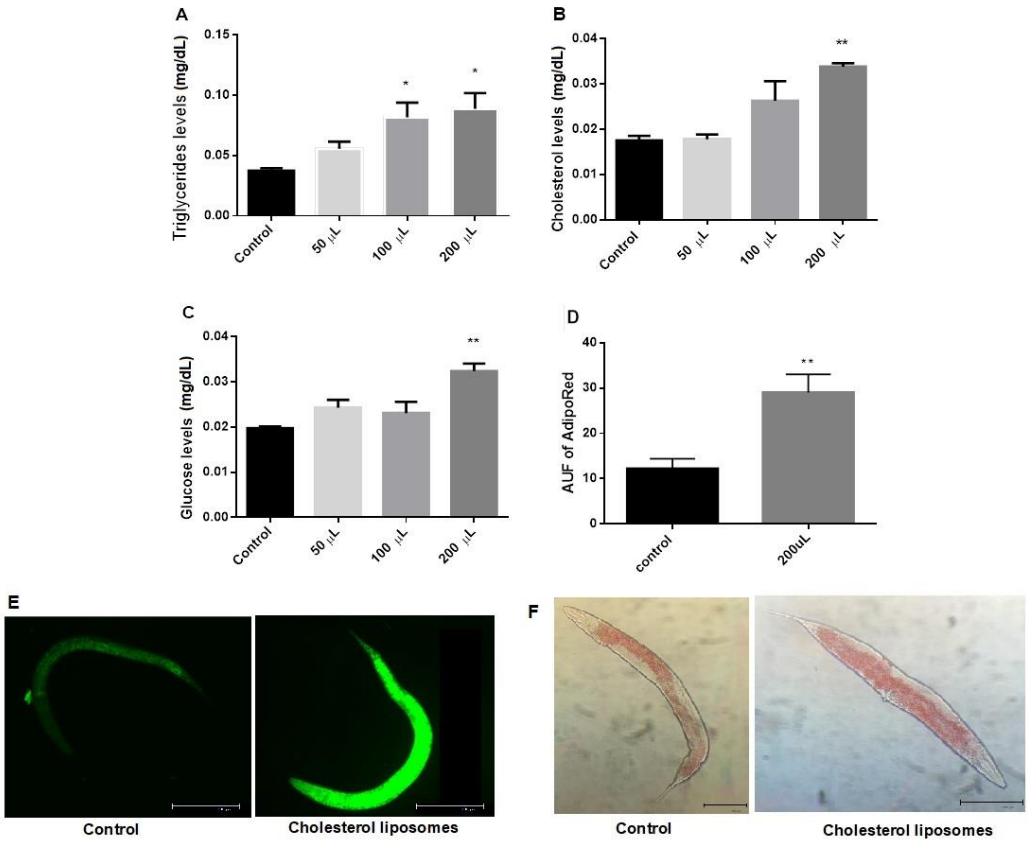


Figure 2. Effect of treatment of liposomes with cholesterol on the levels of (A) triglycerides, (B) cholesterol, (C) glucose, (D) total lipids, (E) representative AdipoRed photos (100 µm magnification) and (F) Oil Red representative photos in *C. elegans* (10X magnification) Values are mean ± standard error. *p<0.05 represent differences of control. All analyzes were performed in at least three independent experiments with replicates in duplicate.

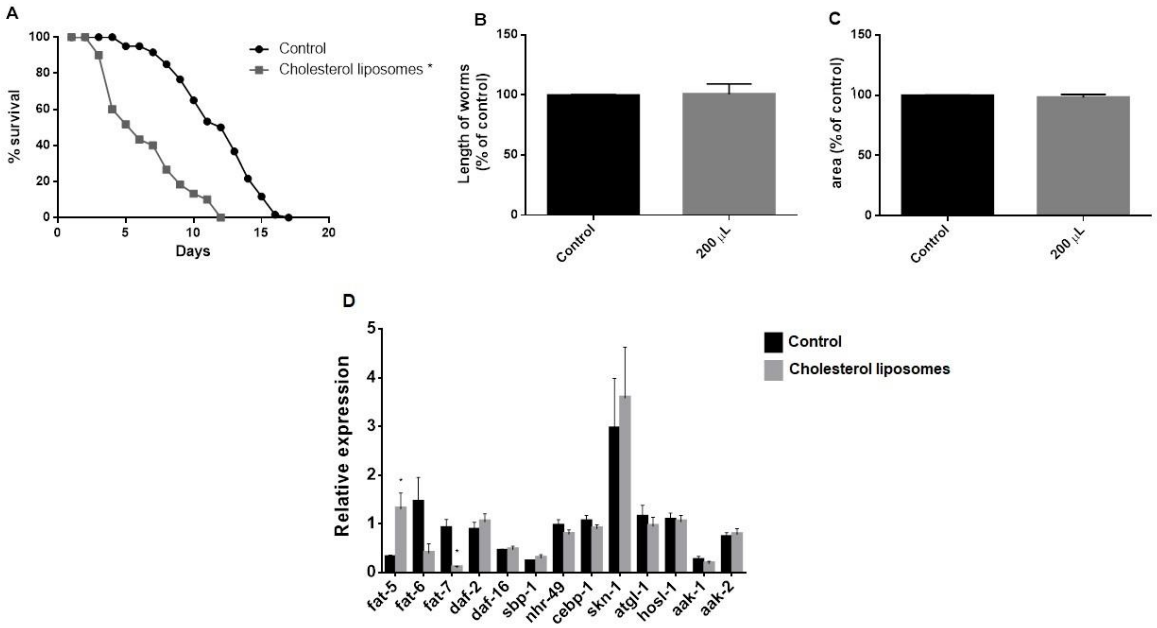


Figure 3. Effect of cholesterol liposome treatment on longevity (A), length (B), total area (C), and relative expression of genes related to lipid metabolism of *C. elegans* (N2 strain) (D). Values are mean ± standard error. *p<0.05 represent differences of control. All analyzes were performed in at least three independent experiments with replicates in duplicate.

4.3 Manuscrito 2

O manuscrito está apresentado da mesma forma que será submetido à revista Food Chemistry.

Extract of purple pitanga fruit (*Eugenia uniflora* L.) regulates fat metabolism and increases longevity in *Caenorhabditis elegans*

Andréia Limana Tambara [1], Cristiane Freitas Rodrigues [1], Jean Ramos Boldori[1], Camila Vieira, Paulo Bayard Gonçalves, Cristiane Casagrande Denardin[1*].

[1] Universidade Federal do Pampa (Unipampa), BR 472, Km 585, Caixa Postal 118, Uruguaiana, CEP 97501-970, RS, Brazil.

*****Corresponding author.

Cristiane Casagrande Denardin. Address: Universidade Federal do Pampa – Campus Uruguaiana. BR 472 - Km 592 - Caixa Postal 118 - Uruguaiana – RS – Brazil – CEP: 97500-970. Phone: +55 55 3413 4321. E-mail: cristianedenardin@unipampa.edu.br

Abstract

Obesity increases the risk of several other chronic diseases and, due to its epidemic proportions, has become an important public health problem worldwide. This work aimed to evaluate the effects of the purple pitanga fruit extract on obesity using the *Caenorhabditis elegans* model fed with a hypercaloric diet. When the wild worms were treated with the purple pitanga fruit extract, after cultivation in a medium with a high cholesterol content, the worms showed decreased levels of triglycerides, cholesterol, total lipids and glucose, in addition to an increase in longevity. In the food consumption test, we did not observe any significant change in food intake. Triglyceride levels in the RB1600 strain (*tub-1* mutant) also decreased. Taken together, these results indicate that the purple pitanga fruit extract plays an important role in reducing fat deposition, modulating cellular pathways for lipid accumulation and prolonging the lifespan of *C. elegans*.

Keywords: Obesity, *C. elegans*, Triglyceride, Cholesterol.

1. Introduction

Obesity increases the risk of several other chronic diseases and, due to its epidemic proportions, has become an important public health problem worldwide (Stefan *et al.*, 2018). Obesity is characterized by the chronic accumulation of regionalized or generalized fatty tissue, resulting from a combination of genetic, environmental and behavioral factors. Excess weight is strongly correlated with the development of metabolic syndrome and other chronic diseases, including type 2 diabetes, cardiovascular disease and hypertension (Loos e Yeo, 2022). Thus increasing the risk of orthopedic, neuroendocrine, pulmonary, gastrointestinal disorders, and psychosocial consequences (Machado *et al.*, 2016). Such relationships highlight the problem of overweight and suggest the urgency and specificity of therapeutic and preventive actions.

Among the protective factors for overweight, the consumption of fruits and vegetables stands out, which have low caloric and fat content, and a high percentage of fiber, contributing to increased satiety and reduced total food intake. , in addition to presenting several bioactive compounds with varied health effects (Machado *et al.*, 2016). Therefore, the search for new compounds or plants that have therapeutic potential for the prevention and/or treatment of obesity is always of great interest (Oliveira *et al.*, 2017).

The pitanga, fruit of the pitangueira (*Eugenia uniflora* L.), belongs to the botanical family of *Myrtaceae*. It is a fruit plant native to Brazil and widely distributed in South America (Bezerra, 2000). Pitanga is a source of fiber, vitamins A and C, minerals, phenolic compounds and carotenoids (De Oliveira Raphaelli *et al.*, 2022). Studies with the extracts of its leaves have shown numerous actions such as antioxidant and anti-inflammatory effects, reduction of triglycerides, reduction of weight

gain and decrease in blood glucose levels (Oliveira *et al.*, 2017). However, the lack of studies related to the pharmacological properties of the fruits of *E. uniflora*, make this fruit a source of bioactive compounds, desirable for obese patients and still little explored.

Caenorhabditis elegans (C. elegans) is an excellent model organism for studying lipid metabolism because it has a relatively short life cycle, a transparent body, a known cell lineage, and a fully sequenced genome*. C. elegans* allows researchers to breed large numbers of inbred animals in short periods of time to perform high-throughput genetic screenings to study a wide range of genes and metabolic pathways (Escorcia *et al.*, 2018).

Therefore, in view of the importance of the search for new therapeutic possibilities for the problem of obesity, further studies are needed to evaluate the effect of consuming the extract of the purple pitanga fruit, a fruit rich in phenolic compounds of great interest to human health. In this sense, this work aimed to evaluate the effects of consumption of purple pitanga fruit extract on diet-induced obesity in a model of *Caenorhabditis elegans* fed a diet rich in cholesterol.

2. Materials and methods

2.1. Extract preparation

The purple pitanga (*Eugenia uniflora L*.) was harvested in its mature stage at the Brazilian Agricultural Research Corporation - EMBRAPACPACT - Pelotas, RS/Brazil, washed in running water and frozen (−18 °C) prior to transportation. For the extraction of the phenolic compounds, 100 g of frozen fruits without seeds were homogenized with 300 ml of ethanolic solution (95%) in light-protected beaker using

an ultra-turrax mixer for 5 min and then placed in a magnetic stirrer for 30 min. Samples were centrifuged for 5 min at 3000 rpm and the supernatant was recovered. The residue was subjected to the new extraction as described above, the supernatant being mixed to the above. The recovered supernatant was evaporated in a rotary evaporator using temperatures between 40 and 45 °C and vacuum. The extract was resuspended in distilled water. The extract obtained was used for the determinations of total phenolic compounds quantified with Folin Ciocalteu (Swain and Hillis, 1959) using a chlorogenic acid curve as standard (purity > 95%, Sigma). To evaluate the in vivo antioxidant activity of the purple pitanga extract (PPE), the concentrations tested in the nematode *C. elegans* were selected based on previous toxicology studies of our group, and taking into account the amount of total phenolic compounds, which resulted in 96,537.68µg of chlorogenic acid/ml (CAE/ml). Therefore the concentrations used were 100, 250 and 500µg CAE/ml. identification and quantification of phenolic compounds in the extract were already performed in the previous study (Tambara *et al.*, 2018).

2.2. Liposome preparation by ethanol injection method

Liposomes were prepared by a ethanol injection method (Jaafar-Maalej *et al.*, 2010). The phospholipids (80 mg) and cholesterol (40 mg) were dissolved in ethanol. The resulting organic phase was injected from what source a syringe pump in distilled water (10 ml) under magnetic stirring. Spontaneous liposome formation occurred instantaneously as soon as the ethanolic solution was in contact with the distilled water. The liposome suspension was kept under stirring for 10 minutes at room temperature. Thereafter, the ethanol was removed by rotary evaporation (Rotavapor R-144; Buchi) under reduced pressure. Final cholesterol concentration in the formulation 4 mg/ml. Characterization of liposomes were performed in the previous study.

2.3. Maintenance of *C. elegans*

C. elegans strains were kept in an incubator at 20°C in Petri dishes containing NGM (nematode growth media) and *Escherichia coli* OP50 as food source (Brenner, 1974). The strains used were N2 (wild type), RB1600 [tub-1(ok1972) II] and CE541 [sbp-1(ep79) III]. All strains were obtained from the Caenorhabditis Genetics Center (Minesotta, USA). Worms were exposed to treatments at the first larval stage (L1). L1 worms were obtained by a synchronization process, which is based on the disruption of hermaphroditic pregnant worms using a lysing solution (1% NaOCl, 2.4% NaOH) in order to obtain the eggs. After 12-14 hours, the eggs hatch isolated, releasing L1 larva.

2.4. Treatment

Worms L1 larvae obtained by synchronization were exposed to treatments with different concentrations of fruit extract for 30 min in a liquid medium containing 0.5% NaCl and in the absence of food, after they were transferred to plates containing NGM and dead *Escherichia coli* OP50, the liposomes with cholesterol were added to the plates and were exposed for 48 h.

2.5. Survival

After 48 hours of exposure to the treatment (2000 worms), the survival test was performed by counting the live worms. All experiments were performed in duplicate and repeated at least three independent times.

2.6. Reproduction

After 48 hours of exposure to the treatment, the reproduction test was performed by counting the brood size during the entire reproductive cycle the worm. All experiments were performed in duplicate and repeated at least three independent times.

2.7. Lifespan

After 48 hours of chronic treatment, twenty worms in duplicate were transferred individually to new plates seeded with dead *E. coli* OP50 and liposomes (200 µl) of cholesterol. The worms were monitored and transferred to new plates every day until the last worm died. All experiments were performed in duplicate and repeated at least three independent times.

2.8. Triglyceride, cholesterol and glucose levels

The levels of triglycerides, cholesterol and glucose were quantified in the (3000) worms treated as previously described. After 48 hours, in phase L4, the worms will be washed until all bacteria are removed. Then, the worms will be frozen twice, sonicated and centrifuged (1200 rpm 1.5 minutes). 50 µl of the supernatant will be transferred to 96-well microplates, which were quantified using the test kits (Labtest kit). For data normalization, protein will be measured in these samples using the Bradford colorimetric method (Bradford, 1976).

2.9. Determination of total lipid levels

To evaluate the total accumulation of lipids, 48h after the treatments, the (3000) worms were transferred to 96-well plates containing 200 µl of phosphate buffered saline and exposed to 5 µL of AdipoRed assay reagent (Lonza). After 10 minutes of

incubation, the animals' fluorescence was measured on a fluorimeter with excitation at 485 nm and emission at 572 nm. Staining with Oil Red was also performed according to Escorcia, Wilber, *et al*. (2018) (Escorcia *et al.*, 2018). Images of the worms were captured using an Olympus inverted microscope, 10X magnification.

2.10. Pumping rate assay

After 48 hours of exposure to the treatment, five L4 worms were transferred to a new NGM plate seeded with bacteria *E. coli* OP50 and then the pumping movements of the pharynx were scored for 1 min. The assay was performed at least three times.

2.11. Statistical analyses

Analyses were performed using GraphPad Prism version 6 (GraphPad Software). To analyze the results of longevity were applied ANOVA for repeated measures and post-hoc Tukey. The one-way ANOVA analysis was applied to all the other tests, followed by post-hoc Tukey. All $p < 0.05$ values were considered statistically significant. The values expressed in percentage (%) were normalized taking a value of 100% for the control. In all figures, error bars represent the standard error of the mean. All analyzes were performed in triplicate.

3. Results

3.1. The purple pitanga fruit extract did not cause any toxic effect on *C. elegans* and increased its longevity

To evaluate the effect of purple pitanga extract together with cholesterol-containing liposomes, we first determined whether any toxic effects would be observed on the nematode *C. elegans*. We observed that increasing concentrations of purple

pitanga extract did not significantly alter worm survival (Fig. 1A). In addition, no changes were observed in the reproduction of the worms observed by the brood size (Fig. 1B). Notably, concentrations of 100, 250, and 500 µg CAE/mL increased the lifespan of the wild-type worm compared to the control and cholesterol liposome groups (Fig. 1C). Based on these results, we can suggest that the purple pitanga extract did not have toxic effects on the nematode *C. elegans*. Obesity induction with cholesterol liposomes significantly reduced worm longevity (Fig. 1C).

3.2. Purple pitanga fruit extract lowers triglycerides, cholesterol and glucose levels

We evaluated the potential of purple pitanga extract to modulate levels of triglycerides (Fig. 2A), cholesterol (Fig. 2B), glucose (Fig. 2C) and total lipids (Fig. 2D and 2F). We can observe that all tested concentrations of purple pitanga extract (100, 250 and 500 µg CAE/mL) significantly decreased their levels compared to the cholesterol liposomes group.

The obesity model with cholesterol liposomes increased levels of cholesterol, triglycerides, glucose and total lipid content in the worms, demonstrating obesity. The purple pitanga extract was able to revert to control levels all parameters in all tested concentrations.

3.3. These lipid-lowering effects were not due to dietary restriction

To assess whether the effects of the purple pitanga extract were due to reduced food consumption, the *C. elegans* pharyngeal pumping count assay was performed (Fig. 3A), where we can observe that there was no significant difference compared to the control group. That is, all worms, regardless of treatment, feed the same way.

3.4. Effects of purple pitanga fruit extract on lipid metabolism

Two genes involved in fat metabolism were evaluated, where the RB1600 strain used to study obesity has a mutation in the *tub-1* gene. Functional loss of *tub-1* in C. elegans leads to lipid accumulation (Purwakusumah e Andrianto, 2017). When treated with purple pitanga fruit extract (Fig. 3B) it showed a decrease in triglyceride levels in all tested concentrations (100, 250 and 500 µg CAE/mL). The CE541 strain carrying an *sbp-1* deletion allele exhibits a low body fat phenotype (Liang *et al.*, 2010), continued with decreased triglyceride levels after treatment with purple pitanga fruit extract (Fig. 3C).

4. Discussion

The fruits of the pitangueira (*Eugenia uniflora* L.) have pleasant sensory attributes, described as acidic, sweet and exotic flavors and a high content of bioactive compounds with antioxidant effects and potential biological effects (De Oliveira Raphaelli *et al.*, 2022). In the present study, the results indicate that the purple pitanga fruit extract increases the longevity of *C. elegans*. As suggested by our previous work in which purple pitanga fruit protects against oxidative stress and increases lifespan in *Caenorhabditis elegans* via the DAF-16/FOXO pathway (Tambara *et al.*, 2018).

Both in *Caenorhabditis elegans* and in other animals, fat regulation reflects the result of behavioral, physiological and metabolic processes. The ease of experimentation of *C. elegans* has led to the use of this organism to elucidate the complex homeostatic mechanisms that underlie energy balance in intact organisms. The optical advantages of *C. elegans* also offer the possibility of studying the cellular

biological mechanisms of absorption, transport, storage and use of fat (De Almeida Barros *et al.*, 2012).

Cholesterol is known to be a component of membrane structures. In addition to this structural activity, cholesterol and its metabolites (steroid hormones, oxysterols, vitamin D and bile acids) are involved in several signaling processes. C. elegans cannot re-synthesize cholesterol and therefore requires dietary cholesterol, which provides a good model for studying fat accumulation similar to the phenotype seen in obesity. Cholesterol and its metabolites have been reported to be involved in the development, germline maturation, and lifespan of worms (Cheong *et al.*, 2011; Ihara *et al.*, 2017).

Our results also demonstrate that purple pitanga fruit extract significantly reduced fat accumulation in wild-type worms without altering food intake, survival and reproduction rates. The purple pitanga fruit extract significantly decreases the triglyceride levels of *tub-1* mutant worms, suggesting its metabolic involvement in fat reduction by this pathway. Tubby proteins, expressed in the human brain and adipose tissue, are involved in a neuroendocrine pathway that regulates many biological pathways, including fatty acid β-oxidation (Farias-Pereira *et al.*, 2020).

Lipid accumulation involves complex processes of lipid biosynthesis, transport and storage (Mak *et al.*, 2006). In *C. elegans*, *tub-1* gene products are believed to manage the accumulation of lipids, which govern beta-oxidation of fatty acids. Critical to the utilization of stored lipids, beta-oxidation converts fatty acids to acetyl co-A and requires the release of fatty acids from triacylglycerides by lipases. (Kim *et al.*, 2010).

Energy homeostasis depends on proper control of the balance between fat synthesis and oxidation. A strain carrying an *sbp-1* deletion allele (*ep79*) results in worms that grow slowly and have decreased fat stores and altered fatty acid

composition compared to the wild-type, indicating that this deletion is a reduced-function allele (Yang *et al.*, 2006; Liang *et al.*, 2010). Treatment with purple pitanga fruit extract suggests that the lower fat accumulation was due to the higher level of beta-oxidation in *C. elegans*. It also that *C. elegans* were shown to be an effective model for in vivo lipid accumulation mechanism and potential to be used as a rapid screening assay for bioactive compounds with lipid accumulation inhibitory activity.

5. Conclusion

Our results demonstrate that the consumption of cholesterol-rich liposomes increased the accumulation of fat and cholesterol in the worms, indicating a phenotype similar to that observed in obesity in other living organisms, and that the consumption of the extract of the purple pitanga fruit promoted a significant reduction of all these evaluated parameters, in addition to increasing the life expectancy of *C. elegans*. Furthermore, we observed that some classic pathways involved in obesity, such as the pathways modulated by the *tub-1* and *sbp-1* genes, may be related to the observed effects. This study shows the promising use of the purple pitanga fruit for the prevention and control of obesity and its potential for pharmaceutical and food purposes.

References

Bezerra, J. E. (2000). *Frutas nativas pitanga (Eugenia uniflora L.)*: Funep.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem, 72*, 248-254.

Brenner, S. (1974). The genetics of Caenorhabditis elegans. *Genetics, 77*(1), 71-94.

Cheong, M. C., Na, K., Kim, H., Jeong, S.-K., Joo, H.-J., Chitwood, D. J., & Paik, Y.-K. (2011). A potential biochemical mechanism underlying the influence of sterol

deprivation stress on Caenorhabditis elegans longevity. *Journal of Biological Chemistry, 286*(9), 7248-7256.

de Almeida Barros, A. G., Liu, J., Lemieux, G. A., Mullaney, B. C., & Ashrafi, K. (2012). Analyses of C. elegans fat metabolic pathways. In *Methods in cell biology*, vol. 107 (pp. 383-407): Elsevier.

de Oliveira Raphaelli, C., Vinholes, J. R., Ribeiro, J. A., Fiorentini, Â. M., Nora, L., & Vizzotto, M. (2022). Eugenia uniflora L. fruit: A review on its Chemical Composition and Bioactivity. *The Natural Products Journal, 12*(2), 42-59.

Escorcia, W., Ruter, D. L., Nhan, J., & Curran, S. P. (2018). Quantification of lipid abundance and evaluation of lipid distribution in Caenorhabditis elegans by nile red and oil red O staining. *JoVE (Journal of Visualized Experiments)*(133), e57352.

Farias-Pereira, R., Park, C.-S., & Park, Y. (2020). Kahweol Reduces Food Intake of Caenorhabditis elegans. *J Agric Food Chem, 68*(36), 9683-9689.

Ihara, A., Uno, M., Miyatake, K., Honjoh, S., & Nishida, E. (2017). Cholesterol regulates DAF-16 nuclear localization and fasting-induced longevity in C. elegans. *Exp Gerontol, 87*, 40-47.

Jaafar-Maalej, C., Diab, R., Andrieu, V., Elaissari, A., & Fessi, H. (2010). Ethanol injection method for hydrophilic and lipophilic drug-loaded liposome preparation. *Journal of liposome research, 20*(3), 228-243.

Kim, H. M., Do, C.-H., & Lee, D. H. (2010). Characterization of taurine as anti-obesity agent in C. elegans. *Journal of biomedical science, 17*(1), 1-6.

Liang, B., Ferguson, K., Kadyk, L., & Watts, J. L. (2010). The role of nuclear receptor NHR-64 in fat storage regulation in Caenorhabditis elegans. *Plos One, 5*(3), e9869.

Loos, R. J., & Yeo, G. S. (2022). The genetics of obesity: from discovery to biology. *Nature Reviews Genetics, 23*(2), 120-133.

Machado, R. H. V., Feferbaum, R., & Leone, C. (2016). Fruit intake and obesity Fruit and vegetables consumption and obesity in Brazil. *Journal of Human Growth and Development, 26*, 243-252.

Mak, H. Y., Nelson, L. S., Basson, M., Johnson, C. D., & Ruvkun, G. (2006). Polygenic control of Caenorhabditis elegans fat storage. *Nature genetics, 38*(3), 363-368.

Oliveira, P. S., Chaves, V. C., Bona, N. P., Soares, M. S. P., de Souza Cardoso, J., Vasconcellos, F. A., Tavares, R. G., Vizzotto, M., da Silva, L. M. C., & Grecco,

F. B. (2017). Eugenia uniflora fruit (red type) standardized extract: a potential pharmacological tool to diet-induced metabolic syndrome damage management. *Biomedicine & pharmacotherapy, 92*, 935-941.

Purwakusumah, E., & Andrianto, D. (2017). In Vivo Inhibition of Lipid Accumulation in Caenorhabditis elegans. In *IOP Conference Series: Earth and Environmental Science*, vol. 58 (pp. 012067): IOP Publishing.

Stefan, N., Häring, H.-U., & Schulze, M. B. (2018). Metabolically healthy obesity: the low-hanging fruit in obesity treatment? *The lancet Diabetes & endocrinology, 6*(3), 249-258.

Tambara, A. L., Moraes, L. d. L. S., Dal Forno, A. H., Boldori, J. R., Soares, A. T. G., de Freitas Rodrigues, C., Mariutti, L. R. B., Mercadante, A. Z., de Ávila, D. S., & Denardin, C. C. (2018). Purple pitanga fruit (Eugenia uniflora L.) protects against oxidative stress and increase the lifespan in Caenorhabditis elegans via the DAF-16/FOXO pathway. *Food and Chemical Toxicology, 120*, 639-650.

Yang, F., Vought, B. W., Satterlee, J. S., Walker, A. K., Jim Sun, Z.-Y., Watts, J. L., DeBeaumont, R., Mako Saito, R., Hyberts, S. G., & Yang, S. (2006). An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. *Nature, 442*(7103), 700-704.

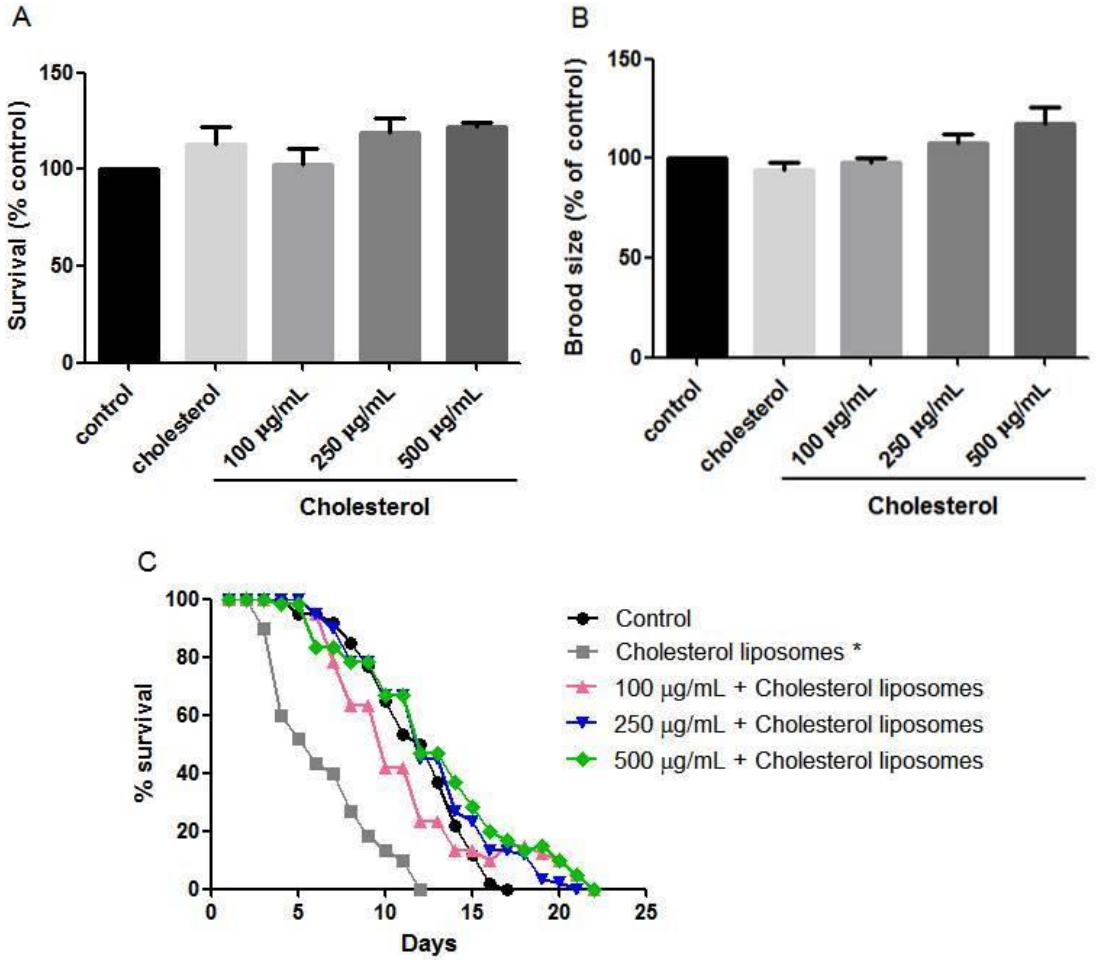


Figure 1. Purple pitanga fruit extract extends lifespan of *C. elegans*. (A) Survival; (B) Brood size; (C) lifespan with N2 (wild type). Values are mean ± standard error of 3 independent experiments. * p <0.05 represent differences in relation to the control.

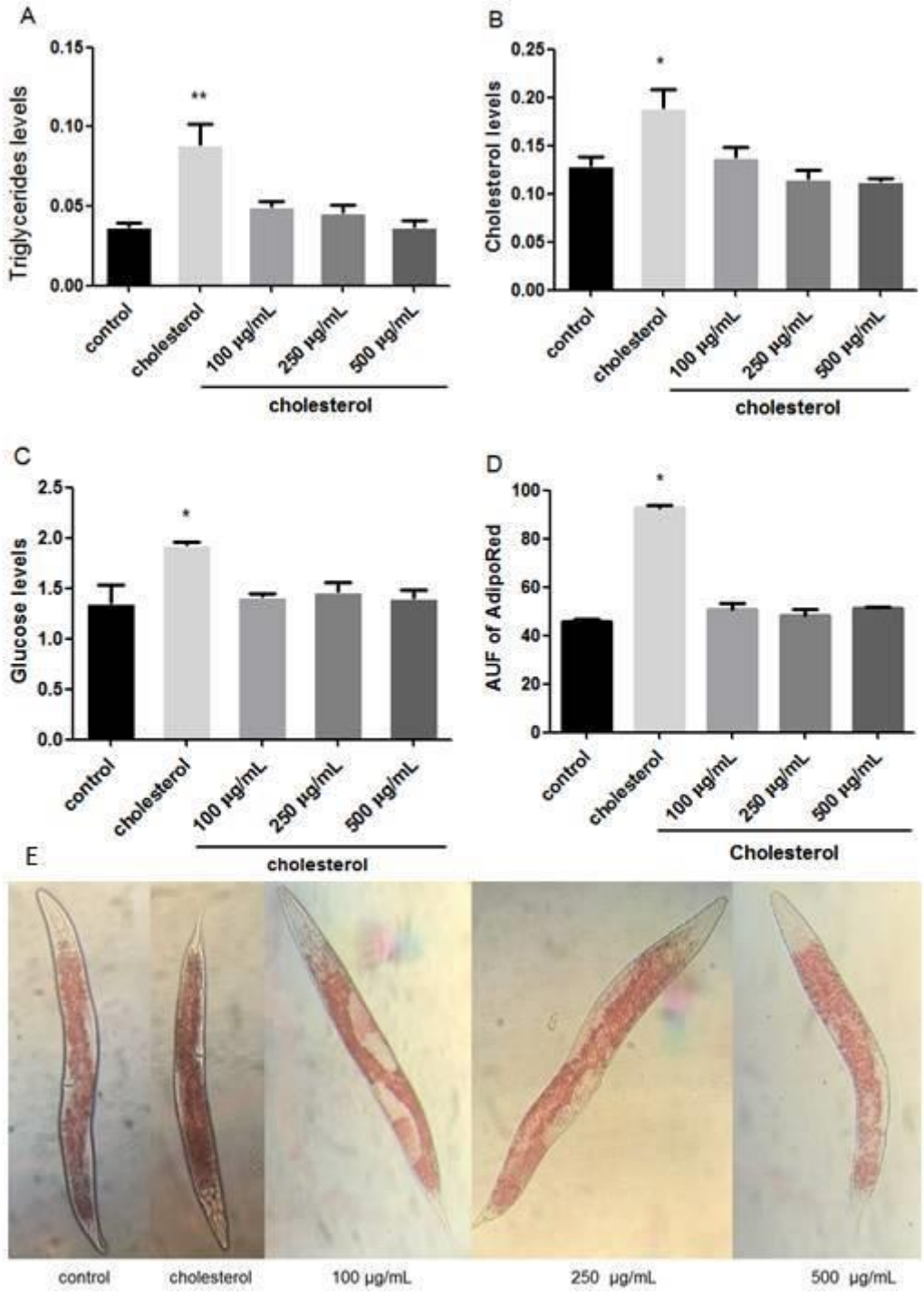


Figure 2. Effects of treatment with purple pitanga fruit extract on the levels of (A) triglycerides, (B) cholesterol, (C) glucose, (D) total lipids, (E) Oil Red representative photos in *C. elegans*. Values are mean ± standard error of 3 independent experiments. * $p < 0.05$ represent differences in relation to the control.

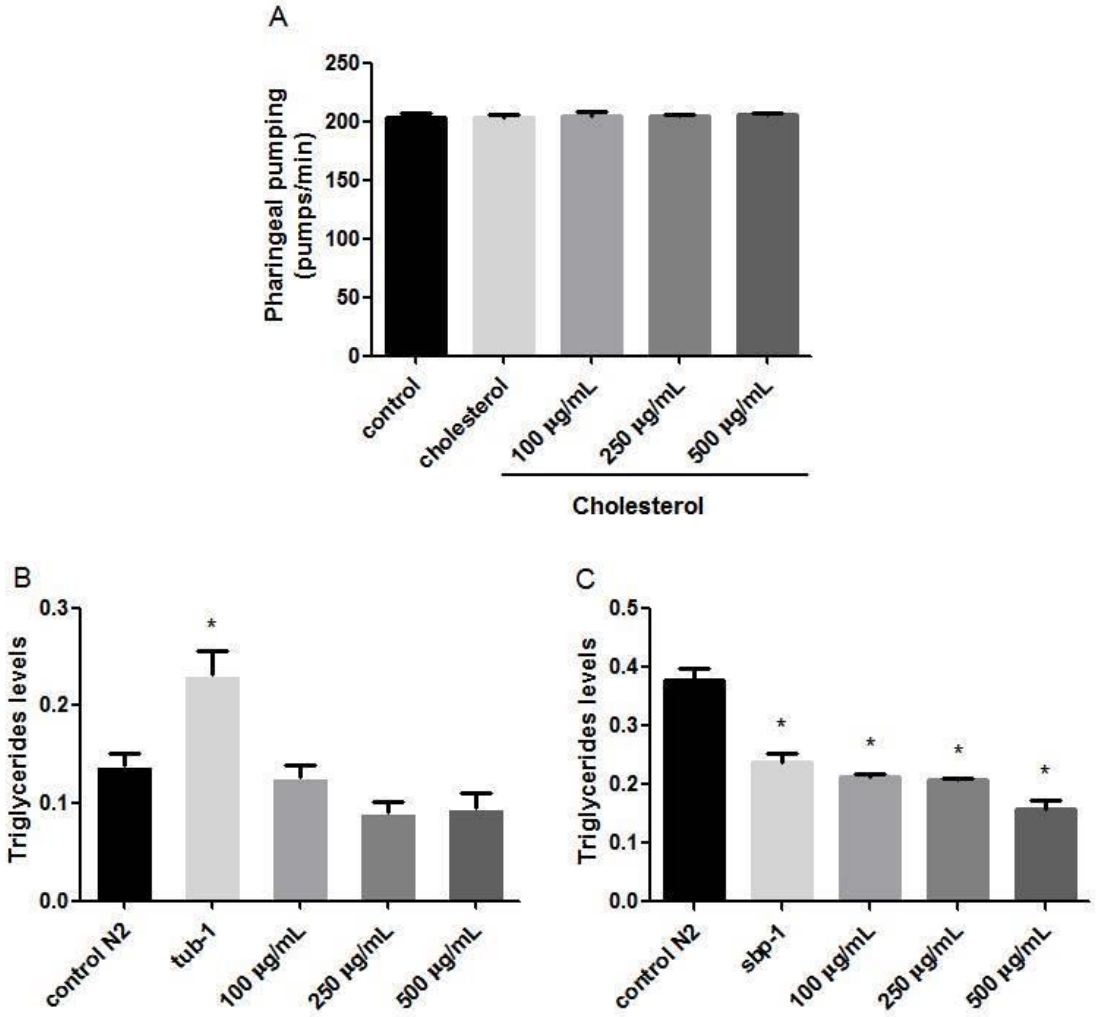


Figure 3. (A) Pharyngeal pumping in N2. Effect of treatment with the purple pitanga fruit extract on triglyceride levels in the strains (B) RB1600 [tub-1(ok1972) II] and (C) CE541 [sbp-1(ep79) III]. Values are mean ± standard error of 3 independent experiments. * p <0.05 represent differences in relation to the control.

5 CONSIDERAÇÕES FINAIS

Os nossos resultados demonstram, que o extrato de butiá tem capacidade de estender o tempo de vida no nematoide *C*. *elegans* e que este efeito pode ser mediado por resistência ao estresse oxidativo. O extrato de butiá também foi capaz de reverter o dano oxidativo induzido pelo peróxido de hidrogênio. A partir dos resultados observa-se que o extrato de butiá não apresentou efeitos tóxicos no nematoide *C. elegans* sendo saudável a sua utilização e demonstrando que os extratos de frutas inteiras têm ótima eficiência.

Também podemos concluir que o modelo de indução de obesidade utilizando lipossomas contendo colesterol pode ser utilizado para aumentar os níveis de lipídios nos *C. elegans*, semelhante à obesidade induzida pela dieta nos animais. Os lipossomas desenvolvidos ficaram em um tamanho adequado, apresentando um consumo adequado pelo verme. Não observamos efeitos tóxicos dos lipossomas com colesterol no nematoide *C. elegans*. Indicando que esta metodologia pode ser utilizada como modelo de indução de obesidade oral em *C. elegans*.

Além disso, podemos concluir que o extrato da fruta pitanga roxa desempenha um papel importante na redução da deposição de gordura, modulando as vias celulares para o acúmulo de lipídios e prolongando a vida útil do *C. elegans*. Sugerindo assim o uso promissor da fruta pitanga roxa para a prevenção e controle da obesidade e seu potencial para fins farmacêuticos e alimentícios.

REFERÊNCIAS

ADENEYE, A. A.; ADEYEMI, O. O.; AGBAJE, E. O. Anti-obesity and antihyperlipidaemic effect of Hunteria umbellata seed extract in experimental hyperlipidaemia. **J Ethnopharmacol,** v. 130, n. 2, p. 307-314, 2010. ISSN 0378-8741.

AGUDO, A. et al. Fruit and vegetable intakes, dietary antioxidant nutrients, and total mortality in Spanish adults: findings from the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). **Am J Clin Nutr,** v. 85, n. 6, p. 1634-42, Jun 2007. ISSN 0002-9165 (Print)

0002-9165 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17556703 >.

ALICE, C. et al. Screening of plants used in south Brazilian folk medicine. **J Ethnopharmacol,** v. 35, n. 2, p. 165-171, 1991. ISSN 0378-8741.

AMIGONI, L. et al. Green coffee extract enhances oxidative stress resistance and delays aging in Caenorhabditis elegans. **Journal of functional foods,** v. 33, p. 297-306, 2017. ISSN 1756-4646.

ARAI, I. et al. Improving effects of the extracts from Eugenia uniflora on hyperglycemia and hypertriglyceridemia in mice. **J Ethnopharmacol,** v. 68, n. 1-3, p. 307-14, Dec 15 1999. ISSN 0378-8741 (Print)

0378-8741 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10624893 >.

ARMANI, A. et al. Cellular models for understanding adipogenesis, adipose dysfunction, and obesity. **Journal of cellular biochemistry,** v. 110, n. 3, p. 564-572, 2010. ISSN 0730-2312.

BAGETTI, M. et al. Physicochemical characterization and antioxidant capacity of pitanga fruits (Eugenia uniflora L.). **Food Science and Technology (Campinas),** v. 31, n. 1, p. 147-154, 2011. ISSN 0101-2061.

BARBOSA, K. B. F. et al. Estresse oxidativo: conceito, implicações e fatores modulatórios. **Revista de Nutrição,** v. 23, p. 629-643, 2010. ISSN 1415-5273. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1415-52732010000400013&nrm=iso >.

BARBOSA, M. C. D. A. et al. Composition proximate, bioactive compounds and antioxidant capacity of Butia capitata. **Food Science and Technology,** v. 41, p. 763-764, 2021. ISSN 0101-2061.

BERNT, U. et al. Effects of anthelminthics with different modes of action on the behavior and development of Caenorhabditis elegans. **Fundamental and applied nematology,** v. 21, n. 3, p. 251-263, 1998. ISSN 1164-5571.

BEZERRA, J. E. **Frutas nativas pitanga (Eugenia uniflora L.)**. Funep, 2000. ISBN 8587632051.

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,** v. 72, p. 248-54, May 7 1976. ISSN 0003-2697 (Print)

0003-2697 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/942051 >.

BRENNER, S. The genetics of Caenorhabditis elegans. **Genetics,** v. 77, n. 1, p. 71-94, May 1974. ISSN 0016-6731 (Print)

0016-6731 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/4366476 >.

CABRERA, M. A.; JACOB FILHO, W. Obesidade em idosos: prevalência, distribuição e associação com hábitos e co-morbidades. **Arquivos Brasileiros de Endocrinologia & Metabologia,** v. 45, n. 5, p. 494-501, 2001. ISSN 0004-2730.

CAMPBELL, C. Cultivation of tropical fruits of the Myrtaceae in southern Florida. Proceedings of the Tropical Region, American Society for Horticultural Science, 1977. p.3-7.

CAMPOS, V. C. et al. Fatores associados ao consumo adequado de frutas, legumes e verduras em adultos de Florianópolis. **Rev. bras. epidemiol,** v. 13, n. 2, p. 352-362, 2010. ISSN 1415-790X.

CARVALHO, P. Butiá-da-serra: Butia eriospatha. 2014.

CAZARIN, K. C. C.; CORRÊA, C. L.; ZAMBRONE, F. A. D. Redução, refinamento e substituição do uso de animais em estudos toxicológicos: uma abordagem atual. **Revista Brasileira de Ciências Farmacêuticas,** v. 40, n. 3, p. 289-299, 2004. ISSN 1809-4562.

CELLI, G. B.; PEREIRA-NETTO, A. B.; BETA, T. Comparative analysis of total phenolic content, antioxidant activity, and flavonoids profile of fruits from two varieties of Brazilian cherry (Eugenia uniflora L.) throughout the fruit developmental stages. **Food research international,** v. 44, n. 8, p. 2442-2451, 2011. ISSN 0963-9969.

CÉSPEDES, C. L. et al. Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry Aristotelia chilensis (Elaeocarpaceae), Maqui. **Food Chem,** v. 107, n. 2, p. 820-829, 2008. ISSN 0308-8146.

CHEONG, M. C. et al. A potential biochemical mechanism underlying the influence of sterol deprivation stress on Caenorhabditis elegans longevity. **Journal of Biological Chemistry,** v. 286, n. 9, p. 7248-7256, 2011. ISSN 0021-9258.

CONSOLINI, A. E.; BALDINI, O. A.; AMAT, A. G. Pharmacological basis for the empirical use of Eugenia uniflora L. (Myrtaceae) as antihypertensive. **J Ethnopharmacol,** v. 66, n. 1, p. 33-9, Jul 1999. ISSN 0378-8741 (Print)

0378-8741 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10432205 >.

CORREA, M.; PENNA, L. D. A. Dicionario das plantas uteis do Brasil e das exoticas cultivadas: volume 5. MR. **Rio de Janeiro: Instituto Brasiliero de Desenvolvimento Florestal 687p.-illus.. Por Icones. Geog,** v. 4, 1974.

CRONQUIST, A. The Evolution and Classification of Flowering Plants, Edition 2. 1988.

DAUCHET, L. et al. Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. **J Nutr,** v. 136, n. 10, p. 2588-93, Oct 2006. ISSN 0022-3166 (Print)

0022-3166 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16988131 >.

DE ALMEIDA BARROS, A. G. et al. Analyses of C. elegans fat metabolic pathways. In: (Ed.). **Methods in cell biology**: Elsevier, v.107, 2012. p.383-407. ISBN 0091-679X.

DE LIRA JÚNIOR, J. S. et al. Empresa Pernambucana de Pesquisa Agropecuária-IPA. 2007.

DE OLIVEIRA RAPHAELLI, C. et al. Eugenia uniflora L. fruit: A review on its Chemical Composition and Bioactivity. **The Natural Products Journal,** v. 12, n. 2, p. 42-59, 2022. ISSN 2210-3155.

DE SOUZA, A. G.; FASSINA, A. C.; DE SOUZA SARAIVA, F. R. Compostos bioativos e atividade antioxidante em frutas nativas do Brasil. 2018.

DENARDIN, C. C. et al. Antioxidant capacity and bioactive compounds of four Brazilian native fruits. **Journal of Food and Drug Analysis,** v. 23, n. 3, p. 387-398, Sep 2015. ISSN 1021-9498. Disponível em: < <Go to ISI>://000361256900006 >.

DENARDIN, C. C. et al. Antiproliferative and cytotoxic effects of purple pitanga (Eugenia uniflora L.) extract on activated hepatic stellate cells. **Cell Biochem Funct,** v. 32, n. 1, p. 16-23, Jan 2014. ISSN 1099-0844 (Electronic)

0263-6484 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23475531 >.

DIOGO, A. D.; MOTA, M. M. *Caenorhabditis elegans*: modelo biológico para o século XXI. . **Biologias**, 2001. Disponível em: < http://arquivo.ordembiologos.pt/Publicacoes/Biologias/1_Caenorhabditis_elegans%20--%2020Abr05.pdf >.

DONÁDIO, L. **Fruticultura para pomares domésticos**. FCAV, 1983.

ESCORCIA, W. et al. Quantification of lipid abundance and evaluation of lipid distribution in Caenorhabditis elegans by nile red and oil red O staining. **JoVE (Journal of Visualized Experiments)**, n. 133, p. e57352, 2018. ISSN 1940-087X.

FARIAS-PEREIRA, R.; PARK, C.-S.; PARK, Y. Kahweol Reduces Food Intake of Caenorhabditis elegans. **J Agric Food Chem,** v. 68, n. 36, p. 9683-9689, 2020. ISSN 0021-8561.

FERREIRA, F. **Espécies frutíferas pouco exploradas, com potencial econômico e social para o Brasil**. Embrapa-CPATU,[19..], 1987.

FIGUEIREDO, I. C. R.; JAIME, P. C.; MONTEIRO, C. A. Fatores associados ao consumo de frutas, legumes e verduras em adultos da cidade de São Paulo. **Rev Saude Publica,** v. 42, n. 5, p. 777-785, 2008. ISSN 1518-8787.

FLYNN, L.; WOODHOUSE, K. A. Adipose tissue engineering with cells in engineered matrices. **Organogenesis,** v. 4, n. 4, p. 228-235, 2008. ISSN 1547-6278.

FOUQUÉ, A. Les plantes médicinales présentes en Fôret Guyanaise. **Fruits,** v. 36, n. 4, p. 223-240, 1981. ISSN 0248-1294.

FRANÇA, B. K. et al. Peroxidação lipídica e obesidade: Métodos para aferição do estresse oxidativo em obesos. **GE jornal português de gastrenterologia,** v. 20, n. 5, p. 199-206, 2013. ISSN 0872-8178.

GARCIA-SANCHO, M. From the genetic to the computer program: the historicity of 'data' and 'computation' in the investigations on the nematode worm C. elegans (1963-1998). **Stud Hist Philos Biol Biomed Sci,** v. 43, n. 1, p. 16-28, Mar 2012. ISSN 1879-2499 (Electronic)

1369-8486 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22326069 >.

GILBERT, S. **Developmental Biology**. Fourth edition. Sinauer Associates Publishers, 1988.

HADDAD, L. J. et al. **Global Nutrition Report 2014: Actions and accountability to accelerate the world s progress on nutrition**. Intl Food Policy Res Inst, 2014. ISBN 0896295648.

HALLIWELL, B.; WHITEMAN, M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? **British journal of pharmacology,** v. 142, n. 2, p. 231-255, 2004. ISSN 0007-1188.

HANL, K.; KIMURA, Y.; OKUDA, H. Anti-obesity effects of natural products. In: (Ed.). **Studies in Natural Products Chemistry**: Elsevier, v.30, 2005. p.79-110. ISBN 1572-5995.

HE, F. J.; NOWSON, C. A.; MACGREGOR, G. A. Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. **Lancet,** v. 367, n. 9507, p. 320-6, Jan 28 2006. ISSN 1474-547X (Electronic)

0140-6736 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16443039 >.

IHARA, A. et al. Cholesterol regulates DAF-16 nuclear localization and fasting-induced longevity in C. elegans. **Exp Gerontol,** v. 87, p. 40-47, 2017. ISSN 0531-5565.

JAAFAR-MAALEJ, C. et al. Ethanol injection method for hydrophilic and lipophilic drug-loaded liposome preparation. **Journal of liposome research,** v. 20, n. 3, p. 228-243, 2010. ISSN 0898-2104.

JADEJA, R. N. et al. Anti-obesity potential of Clerodendron glandulosum. Coleb leaf aqueous extract. **J Ethnopharmacol,** v. 135, n. 2, p. 338-343, 2011. ISSN 0378-8741.

JAIME, P. C. et al. Fatores associados ao consumo de frutas e hortaliças no Brasil, 2006. **Rev Saude Publica,** v. 43, n. suppl 2, p. 57-64, 2009. ISSN 0034-8910.

KEY, T. J. et al. The effect of diet on risk of cancer. **Lancet,** v. 360, n. 9336, p. 861-8, Sep 14 2002. ISSN 0140-6736 (Print)

0140-6736 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12243933 >.

KIM, H. M.; DO, C.-H.; LEE, D. H. Characterization of taurine as anti-obesity agent in C. elegans. **Journal of biomedical science,** v. 17, n. 1, p. 1-6, 2010. ISSN 1423-0127.

KNAI, C. et al. Getting children to eat more fruit and vegetables: a systematic review. **Preventive medicine,** v. 42, n. 2, p. 85-95, 2006. ISSN 0091-7435.

LAHAV, E.; SLOR, E. 'Gitit'a new Surinam cherry cultivar. **Fruit varieties journal (USA)**, 1997. ISSN 0091-3642.

LEDERMAN, I.; BEZERRA, J.; CALADO, G. A pitangueira em Pernambuco. 1992, IPA.

LEE, M. H. et al. EBV DNA polymerase inhibition of tannins from Eugenia uniflora. **Cancer Lett,** v. 154, n. 2, p. 131-6, Jun 30 2000. ISSN 0304-3835 (Print)

0304-3835 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/10806300 >.

LEITE, H. P.; SARNI, R. S. Radicais livres, antioxidantes e nutrição. **Rev Bras Nutr Clin,** v. 18, n. 2, p. 87-94, 2003.

LIANG, B. et al. The role of nuclear receptor NHR-64 in fat storage regulation in Caenorhabditis elegans. **Plos One,** v. 5, n. 3, p. e9869, 2010. ISSN 1932-6203.

LOOS, R. J.; YEO, G. S. The genetics of obesity: from discovery to biology. **Nature Reviews Genetics,** v. 23, n. 2, p. 120-133, 2022. ISSN 1471-0064.

MACHADO, R. H. V.; FEFERBAUM, R.; LEONE, C. Fruit intake and obesity Fruit and vegetables consumption and obesity in Brazil. **Journal of Human Growth and**

Development, v. 26, p. 243-252, 2016. ISSN 0104-1282. Disponível em: < http://pepsic.bvsalud.org/scielo.php?script=sci_arttext&pid=S0104-12822016000200016&nrm=iso >.

MAK, H. Y. et al. Polygenic control of Caenorhabditis elegans fat storage. **Nature genetics,** v. 38, n. 3, p. 363-368, 2006. ISSN 1546-1718.

MENTZ, L. A.; LUTZEMBERGER, L. C.; SCHENKEL, E. P. Da flora medicinal do Rio Grande do Sul: notas sobre a obra de D'Avila (1910). **Caderno de farmácia. Porto Alegre, RS. Vol. 13, n. 1 (jan./jun. 1997), p. 25-47**, 1997. ISSN 0102-6593.

MEZADRI, T. et al. Antioxidant compounds and antioxidant activity in acerola (Malpighia emarginata DC.) fruits and derivatives. **Journal of Food Composition and Analysis,** v. 21, n. 4, p. 282-290, 2008. ISSN 0889-1575.

MONDINI, L. et al. Consumo de frutas e hortaliças por adultos em Ribeirão Preto, SP. **Rev Saude Publica,** v. 44, n. 4, p. 686-694, 2010. ISSN 0034-8910.

MOREUIL, C. Notes brèves sur quelques espèces fruitières de la côte est de Madagascar. **Fruits**, 1971.

MORO, C.; BASILE, G. Obesity and medicinal plants. **Fitoterapia,** v. 71, p. S73-S82, 2000. ISSN 0367-326X.

NEPAL, V. P. et al. Determinants of Fruits and Vegetables Consumption Among Persons With Doctor-Diagnosed Chronic Diseases. **Journal of primary care & community health,** v. 3, n. 2, p. 132-141, 2012. ISSN 2150-1319.

NEUTZLING, M. B. et al. Fatores associados ao consumo de frutas, legumes e verduras em adultos de uma cidade no Sul do Brasil Factors associated with fruit and vegetable intake among adults in a southern. **Cad. Saúde Pública,** v. 25, n. 11, p. 2365-2374, 2009.

OLIVEIRA, P. S. et al. Eugenia uniflora fruit (red type) standardized extract: a potential pharmacological tool to diet-induced metabolic syndrome damage management. **Biomedicine & pharmacotherapy,** v. 92, p. 935-941, 2017. ISSN 0753-3322.

PARK, S. K. Electrolyzed-reduced water increases resistance to oxidative stress, fertility, and lifespan via insulin/IGF-1-like signal in C. elegans. **Biol Res,** v. 46, n. 2, p. 147-52, 2013. ISSN 0717-6287 (Electronic)

0716-9760 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23959012 >.

PEIXOTO, H. et al. An Anthocyanin-rich extract of açai (Euterpe precatoria Mart.) increases stress resistance and retards aging-related markers in Caenorhabditis elegans. **J Agric Food Chem,** v. 64, n. 6, p. 1283-1290, 2016. ISSN 0021-8561.

PIZZIOLO, V. R. et al. Plantas com possível atividade hipolipidêmica: uma revisão bibliográfica de livros editados no Brasil entre 1998 e 2008. **Revista Brasileira de Plantas Medicinais,** v. 13, n. 1, p. 98-109, 2011. ISSN 1516-0572.

POLITZ, S. M.; PHILIPP, M. Caenorhabditis elegans as a model for parasitic nematodes: a focus on the cuticle. **Parasitol Today,** v. 8, n. 1, p. 6-12, Jan 1992. ISSN 0169-4758 (Print)

0169-4758 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15463517 >.

POPENOE, W. **Manual of tropical and subtropical fruits: Excluding the banana, coconut, pineapple, citrus fruits, olive, and fig**. Macmillan, 1920.

PRYNNE, C. J. et al. Fruit and vegetable intakes and bone mineral status: a cross-sectional study in 5 age and sex cohorts. **Am J Clin Nutr,** v. 83, n. 6, p. 1420-1428, 2006. ISSN 0002-9165.

PURWAKUSUMAH, E.; ANDRIANTO, D. In Vivo Inhibition of Lipid Accumulation in Caenorhabditis elegans. IOP Conference Series: Earth and Environmental Science, 2017, IOP Publishing. p.012067.

RAYALAM, S.; DELLA-FERA, M. A.; BAILE, C. A. Phytochemicals and regulation of the adipocyte life cycle. **J Nutr Biochem,** v. 19, n. 11, p. 717-726, 2008. ISSN 0955-2863.

RIVERA, D.; OBON, C. The ethnopharmacology of Madeira and Porto Santo Islands, a review. **J Ethnopharmacol,** v. 46, n. 2, p. 73-93, May 1995. ISSN 0378-8741 (Print)

0378-8741 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7650952 >.

RODRIGUEZ-AMAYA, D. B. et al. Updated Brazilian database on food carotenoids: Factors affecting carotenoid composition. **Journal of Food Composition and Analysis,** v. 21, n. 6, p. 445-463, 2008. ISSN 0889-1575.

ROH, C.; JUNG, U. Screening of crude plant extracts with anti-obesity activity. **International Journal of Molecular Sciences,** v. 13, n. 2, p. 1710-1719, 2012.

SANTOS, K. K. et al. Anti-Trypanosoma cruzi and cytotoxic activities of Eugenia uniflora L. **Experimental Parasitology,** v. 131, n. 1, p. 130-132, 2012. ISSN 0014-4894.

SARTORELLI, D. S.; FRANCO, L. J. Tendências do diabetes mellitus no Brasil: o papel da transição nutricional. **Cadernos de saúde pública,** v. 19, p. S29-S36, 2003. ISSN 0102-311X.

SCHAPOVAL, E. E. et al. Evaluation of some pharmacological activities of Eugenia uniflora L. **J Ethnopharmacol,** v. 44, n. 3, p. 137-42, Dec 1994. ISSN 0378-8741 (Print)

0378-8741 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7898120 >.

SCHMEDA-HIRSCHMANN, G. et al. Preliminary pharmacological studies on Eugenia uniflora leaves: xanthine oxidase inhibitory activity. **J Ethnopharmacol,** v. 21, n. 2, p. 183-186, 1987. ISSN 0378-8741.

SGANZERLA, M. Caracterização físico-química e capacidade antioxidante do butiá. 2010.

SILVA, S. D. M. Pitanga. **Revista Brasileira de Fruticultura,** v. 28, p. 0-0, 2006. ISSN 0100-2945. Disponível em: < http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0100-29452006000100001&nrm=iso >.

SILVEIRA, C. S. et al. Atividade antimicrobiana dos frutos de Syagrus oleracea e Mauritia vinifera. **Revista Brasileira de Farmacognosia,** v. 15, n. 2, p. 143-148, 2005.

SINGH, M. Role of micronutrients for physical growth and mental development. **The Indian journal of pediatrics,** v. 71, n. 1, p. 59-62, 2004. ISSN 0973-7693.

SOUSA, C. M. D. M. et al. Total phenolics and antioxidant activity of five medicinal plants. **Quimica Nova,** v. 30, n. 2, p. 351-355, Mar-Apr 2007. ISSN 0100-4042. Disponível em: < <Go to ISI>://000244752500021 >.

STEFAN, N.; HÄRING, H.-U.; SCHULZE, M. B. Metabolically healthy obesity: the low-hanging fruit in obesity treatment? **The lancet Diabetes & endocrinology,** v. 6, n. 3, p. 249-258, 2018. ISSN 2213-8587.

STURROCK, D. **Fruits for southern Florida**. Horticultural Books, 1972.

SURVESWARAN, S. et al. Antioxidant properties and principal phenolic phytochemicals of Indian medicinal plants from Asclepiadoideae and Periplocoideae. **Nat Prod Res,** v. 24, n. 3, p. 206-21, Feb 2010. ISSN 1478-6427 (Electronic)

1478-6419 (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20140800 >.

TAMBARA, A. L. et al. Purple pitanga fruit (Eugenia uniflora L.) protects against oxidative stress and increase the lifespan in Caenorhabditis elegans via the DAF-16/FOXO pathway. **Food and Chemical Toxicology,** v. 120, p. 639-650, 2018. ISSN 0278-6915.

VAYNDORF, E. M.; LEE, S. S.; LIU, R. H. Whole apple extracts increase lifespan, healthspan and resistance to stress in Caenorhabditis elegans. **Journal of functional foods,** v. 5, n. 3, p. 1235-1243, 2013. ISSN 1756-4646.

VELAZQUEZ, E. et al. Antioxidant activity of Paraguayan plant extracts. **Fitoterapia,** v. 74, n. 1-2, p. 91-7, Feb 2003. ISSN 0367-326X (Print)

0367-326X (Linking). Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12628400 >.

VELIOGLU, Y. et al. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. **J Agric Food Chem,** v. 46, n. 10, p. 4113-4117, 1998. ISSN 0021-8561.

VELLOSA, J. C. R. et al. Estresse oxidativo: uma introdução ao estado da arte. **Brazilian Journal of Development,** v. 7, n. 1, p. 10152-10168, 2021. ISSN 2525-8761.

VIGITEL, B. **Vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico [acesso em 29 ago 2019]** 2018.

WANG, H. C., G.;; PRIOR, R. L. Total antioxidant capacity of fruits. **Journal of Agricultural and Food Chemistry,** v. 44, p. 701-705, 1996.

WHO. Fruit and vegetables for health : report of the Joint FAO/WHO Workshop on Fruit and Vegetables for Health, 1-3 September 2004, Kobe, Japan. **Geneva: World**

Health Organization, 2004. Disponível em: < http://apps.who.int/iris/bitstream/10665/43143/1/9241592818_eng.pdf >.

WHO, J.; CONSULTATION, F. E. Diet, nutrition and the prevention of chronic diseases. **World Health Organ Tech Rep Ser,** v. 916, n. i-viii, 2003.

WOOD, W. B. Determination of pattern and fate in early embryos of Caenorhabditis elegans. **Dev Biol (N Y 1985),** v. 5, p. 57-78, 1988. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/3077982 >.

YANG, F. et al. An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. **Nature,** v. 442, n. 7103, p. 700-704, 2006. ISSN 1476-4687.