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Marina Lopes Machado

EFEITO DA EXPOSIÇÃO CRÔNICA DO EXTRATO AQUOSO DE *Ilex paraguariensis* SOBRE O COMPORTAMENTO E O PERFIL LIPÍDICO EM *Caenorhabditis elegans*

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Dissertação apresentada ao Curso de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Ciências Biológicas: Bioquímica Toxicológica.**

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Aprovado em 25 de fevereiro de 2016:

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(Chico Xavier)

APRESENTAÇÃO

No item INTRODUÇÃO consta uma revisão sucinta da literatura sobre os temas trabalhados nesta dissertação.

A metodologia realizada e os resultados obtidos que fazem parte desta dissertação estão apresentados no item MANUSCRITO sob a forma de um manuscrito redigido em inglês conforme as normas do periódico ao qual foi submetido. No mesmo constam as seções: Introdução, Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas.

Os itens CONCLUSÕES e PERSPECTIVAS, encontrados no final desta dissertação, apresentam conclusões gerais sobre os resultados do manuscrito presente neste trabalho e as perspectivas para futuros trabalhos.

As REFERÊNCIAS BIBLIOGRÁFICAS referem-se somente às citações que aparecem no item INTRODUÇÃO desta dissertação.

RESUMO

EFEITO DA EXPOSIÇÃO CRÔNICA DO EXTRATO AQUOSO DE *Ilex paraguariensis* SOBRE O COMPORTAMENTO E O PERFIL LIPÍDICO EM *Caenorhabditis elegans*

AUTOR: Marina Lopes Machado
ORIENTADOR: Prof. Dr. Félix Alexandre Antunes Soares

Ilex paraguariensis é uma planta amplamente consumida no sul do Brasil, Uruguai e Argentina, e bem caracterizada por suas propriedades antioxidantes e estimulantes, e pode ser usada como um modulador dos depósitos de gordura para o controle da obesidade, um problema mundial. Portanto, o efeito do consumo crônico de *Ilex paraguariensis* no metabolismo de gordura foi investigado usando o nematódeo *Caenorhabditis elegans.* Primeiramente, foi realizado a identificação e quantificação por DAD-HPLC, dos constituintes do extrato aquoso de *Ilex paraguariensis*. Posteriormente, os nematódeos foram tratados com 1mg/mL de *I. paraguariensis* a partir do estágio L1 até a vida adulta. A concentração utilizada do extrato foi escolhida baseada em suas propriedades antioxidantes contra juglone, um gerador endógeno de espécies reativas de oxigênio, uma vez que estas estão aumentadas no tecido adiposo de pessoas obesas. O acumulo de lipídeos, o gasto total de energia e alguns parâmetros comportamentais foram analisados. *Ilex paraguariensis* diminuiu a fluorescência do corante BODIPY em 63,36% comparado ao controle sem afetar os comportamentos relacionados ao balanço energético. O gasto energético total da cepa selvagem N2, das mutantes *nhr-49* e *ador-1* foi quantificado através do consumo de oxigênio, *Ilex paraguariensis* aumentou o consumo de O_2 apenas na cepa selvagem. Em conclusão, *Ilex paraguariensis* diminuiu os depósitos de lipídeos e aumentou o consumo de oxigênio na cepa selvagem, de uma forma dependente das vias NHR-49 e ADOR-1.

Palavras chave: metabolismo de gordura; lipídeos; produtos naturais; consumo de oxigênio, erva-mate.

ABSTRACT

EVALUATION OF THE EFFECT OF CHRONIC EXPOSITION TO *Ilex paraguariensis* AQUEOUS EXTRACT ON THE BEHAVIOR AND LIPID PROFILE IN *Caenorhabditis elegans*

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ADVISOR: Félix Alexandre Antunes Soares

Ilex paraguariensis, is a plant widely consumed in southern Brazil, Uruguay and Argentina, and well characterized for its antioxidant and stimulating properties, and could be used as modulator of fat storage in order to control obesity, a worldwide problem. Thus, the effect of chronic consumption of *I. paraguariensis* on fat metabolism was investigated using the nematode *Caenorhabditis elegans*. First was carried out the identification and quantification, by HPLC-DAD, of *Ilex paraguariensis* aqueous extract constituents, later, the nematodes were treated with 1 mg/ml of *I. paraguariensis* from L1-larvae-stage until adulthood. The extract concentration was chosen because exhibited antioxidant proprieties against juglone, a generator of reactive oxygen species, since these are elevated in fat tissue of obese people. Lipid accumulation, total body energy expenditure and behavioral parameters were analyzed. *Ilex paraguariensis* decreased BODIPY labeling by 63.36% compared to control without affecting behaviors related to energetic balance. Total body energy expenditure of N2 wild-type, *nhr-49* and *ador-1* knockout strains was performed through oxygen consumption, *Ilex paraguariensis* was able to increase oxygen consumption only in N2 worms. In conclusion, *Ilex paraguariensis* decreased fat deposits and increased consumption of oxygen in N2 worms dependent on NHR-49 and ADOR-1 pathways.

Keywords: fat metabolism; lipids; natural products; oxygen consumption; *yerba mate*.

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LISTA DE ABREVIATURAS

Introdução

ador-1: Gene que codifica o receptor de adenosina

AMPc: Monofosfato cíclico de adenosina

EG6870 *ador-1(ox489)*: Cepa com perda de função do gene *ador-1*

N2: Cepa selvagem

nhr-49: Gene que codifica o receptor nuclear hormonal

PDE: Fosfodiesterase

STE68 *nhr-49*(nr2041)I: Cepa com perda de função do gene *nhr-49*

Manuscrito

C1-BODIPY-C12: Fluorescently labeled fatty acids human analog

EG6870 *ador-1(ox489)*: Adenosine receptor knockdown

HPLC-DAD: High performance liquid chromatography

LOD: Limit of detection

LOQ: Limit of quantification

nhr-49: nuclear hormone receptor gene

ador-1: adenosine receptor gene

N2: Wild-type *C. elegans* strain

PKA: Protein Kinase A

ROS: Reactive Oxygen Species

STE68 *nhr-49* (nr2041) I: Nuclear hormone receptor NHR-49 knockdown.

TCA: Mitochondrial tricarboxylic acid cycle

SUMÁRIO

1. INTRODUÇÃO

A obesidade tem se tornado cada vez mais um problema social, e nos últimos anos têm chamado atenção de instituições públicas de saúde, uma vez que afeta quase um terço da população mundial. Em 2014, mais de 1,9 bilhão de adultos, com 18 anos ou mais, estavam acima do peso, destes, mais de 600 milhões eram obesos (Shaw, 2014). De acordo com a Organização Mundial de Saúde (OMS), o quadro de sobrepeso e obesidade são definidos quando há um acúmulo anormal ou excessivo de gordura no organismo, ou seja, quando a ingestão energética, principalmente armazenada como triglicerídeos, excede as despesas de energia (Spiegelman e Flier, 2001). A obesidade é uma doença complexa, influenciada pela dieta, gênes, gênero, idade e atividade física (Brockmann e Bevova, 2002), e está comumente associada à algumas doenças metabólicas, à hipertensão, ao diabetes tipo II e à resistência insulínica (Pi-Sunyer, 2004). Além disso, outra consequência do aumento do tecido adiposo é o acúmulo de espécies reativas de oxigênio (EROs) nos adipócitos, que quando em excesso, pode gerar estresse oxidativo sistêmico (Furukawa *et al.*, 2004).

O metabolismo celular produz continuamente EROs como subprodutos da respiração e de algumas atividades enzimáticas (xantina oxidase e ciclooxigenases, por exemplo) e como sistema de defesa em células fagocíticas e de transdução de sinal (Halliwell, 1994; Park *et al.*, 2004; Finkel, 2011). As EROs incluem o ânion superóxido (O_2^-), o radical hidroxil ($OH^\bullet$), o oxigênio singleto ($^1O_2$) e o peróxido de hidrogênio (H_2O_2) (Halliwell e Gutteridge, 1999). Geralmente, as espécies reativas podem ser neutralizadas por antioxidantes enzimáticos (como superóxido dismutase e catalase) e não enzimáticos (como glutationa e vitamina C) (Rodriguez-Martinez *et al.*, 2000; Santamaria *et al.*, 2003). A formação de EROs, em concentrações fisiológicas, é necessária para a função celular normal, mas em quantidades excessivas, pode levar ao estresse oxidativo (Nordberg e Arner, 2001).

O estresse oxidativo ocorre devido ao desequilíbrio entre a produção de EROs e a habilidade do sistema antioxidante em detoxificar os intermediários reativos, ou em reparar os danos em componentes celulares, que podem incluir proteínas, lipídios e DNA (Halliwell e Gutteridge, 1999). Devido aos prejuízos causados às células, o estresse oxidativo é gerado em uma série de doenças como obesidade, aterosclerose, câncer e desordens neurodegenerativas.

Vários métodos são utilizados para tratar a obesidade, a maioria deles são produtos químico-farmacêuticos que podem causar efeitos indesejáveis, como distúrbios psiquiátricos graves, ataque cardíaco e acidente vascular cerebral (Kang e Park, 2012). Uma vez que a

obesidade leva a um acúmulo de EROs no tecido adiposo (Keaney *et al.*, 2003; Furukawa *et al.*, 2004), combinações de antioxidantes naturais são bons candidatos para o desenvolvimento de tratamentos contra a obesidade, pois oferecem uma variedade de mecanismos para reduzir os metabólitos do oxigênio nos tecidos, e podem alterar vias de sinalização, modular fatores de transcrição, e também podem desempenhar papéis-chave na redução dos danos causados pelas EROs nos tecidos.

Em muitos países, terapias que envolvem o uso de medicamentos à base de plantas são popularmente utilizadas como abordagem principal ou complementar a tratamentos medicamentosos. Devido aos efeitos colaterais, ineficácia e elevado custo dos medicamentos alopáticos comumente utilizados para tratar a obesidade (Mayer *et al.*, 2009), a utilização de plantas medicinais tem se tornado uma alternativa à ser explorada pela população, principalmente pelo seu fácil acesso, baixo custo, não exigência de prescrição médica e crença na atoxicidade. Além disso, o uso de plantas medicinais desperta um interesse na indústria farmacêutica, uma vez que podem elas podem ser uma alternativa para o desenvolvimento de futuros medicamentos, incluindo àqueles que levam a redução de peso.

Ilex paraguariensis (St. Hill. Aquifoliaceae), é uma espécie arbórea nativa da América do Sul, amplamente usada para a preparação de uma bebida de sabor amargo e peculiar, feita pela infusão ou decocção de suas partes aéreas, regionalmente conhecida como chimarrão ou tererê (em Português) ou mate (em espanhol) (De Andrade *et al.*, 2012). O extrato apresenta várias vitaminas como E, C, tiamina, niacina, riboflavina, ácido pantotênico e betacaroteno (Bixby *et al.*, 2005), alguns minerais como fósforo, ferro e cálcio (Graham, 1984) e também cafeína, flavonoides, metilxantinas, taninos e diversas saponinas triterpênicas (Pang *et al.*, 2008). Sua composição varia conforme a região em que a planta é cultivada, tipo de solo, água, fertilizantes, processo industrial e condições de estocagem. Além disso, a viabilidade dos componentes de *I. paraguariensis* também depende da solubilidade dos compostos envolvidos, de como é preparado (tempo de extração e temperatura) e ingerido (Giulian *et al.*, 2007).

Diferentes efeitos foram descritos para *I. paraguariensis*, como estimulação do sistema nervoso central (Ito *et al.*, 1997), atividade antioxidante (Filip, R. *et al.*, 2001), ação anti-inflamatória e hipocolesterolêmica (Gnoatto *et al.*, 2005). O extrato também é considerado um potente inibidor de radicais livres (Schinella *et al.*, 2005), tanto *in vivo* (Mosimann *et al.*, 2006) quanto *in vitro* (Gugliucci, 1996). Em relação à toxicidade, um estudo realizado com ratos e coelhos não encontrou efeitos tóxicos para as espécies estudadas

(De Andrade *et al.*, 2012), por fim, no nematódeo *Caenorhabditis elegans*, um estudo realizado com o extrato aquoso de *Ilex paraguariensis*, mostrou que o tratamento crônico aumentou o tempo de vida do nematódeo devido ao potencial antioxidante do extrato (Lima *et al.*, 2014). No entanto, nada se sabe em relação aos efeitos sobre o metabolismo de *C. elegans*.

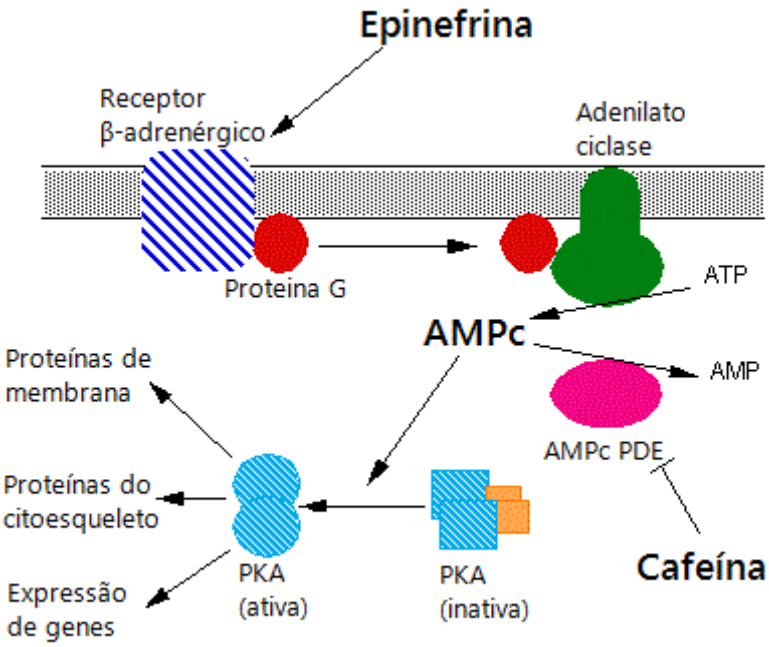
As metilxantinas, cafeína e teobromina são importantes compostos bioativos presente em *I. paraguariensis* (Reginatto et al., 1999), sendo a cafeína, presente em maior quantidade (Bastos *et al.*, 2007). Sua significância biológica é atribuída ao fato de serem alcaloides de purina, uma vez que as bases de purina são os principais componentes de nucleoproteínas e desempenham importante papel em organismos vivos. A ação farmacológica das metilxantinas consiste no bloqueio dos receptores de adenosina A1, A2A, A2B e A3 no sistema nervoso central (SNC), e controlam a liberação de neurotransmissores, a excitabilidade neuronal e o ritmo circadiano (Cunha, 2001; Fredholm *et al.*, 2005).

Os alvos farmacológicos da cafeína são os receptores de adenosina do tipo A1 e A2A (Nehlig *et al.*, 1992; Fredholm *et al.*, 1999), que exercem ações inibitórias e excitatórias, respectivamente, sobre a transmissão sináptica (Cunha, 2001). Além do SNC, o receptor do tipo A1 também está presente no tecido adiposo humano branco e marrom (Linden, 1991), e está envolvido no controle da obesidade. O receptor A1 é uma proteína transmembrana ligada a proteína G, seu principal alvo é a enzima adenilato ciclase (Linden, 1991), a ligação da adenosina ao receptor A1 leva a inibição da adenilato ciclase e a diminuição dos níveis de AMP cíclico (AMPc) no tecido adiposo, e portanto, inibe a lipólise (Challiss *et al.*, 1992). Um estudo realizado com adipócitos de ratos Zucker (fa/fa), uma linhagem de ratos obesos, mostrou que quando incubados com 8-phenyltheophilline, um antagonista do receptor de adenosina tipo A1, a produção de AMPc foi suficiente para aumentar a lipólise nas células obesas (Lanoue e Martin, 1994).

Além disso, outro mecanismo de ação da cafeína, é a inibição direta da enzima fosfodiesterase (PDE), que previne a quebra intracelular de AMPc. O AMPc disponível ativa a proteína kinase A (PKA) (figura 1), que fosforila substratos específicos, como a lipase sensível a hormônio, enzima que ativada, é responsável pelo aumento da lipólise (Nehlig *et al.*, 1992). Estudos demostram que a cafeína aumenta a quantidade de energia disponível, sob a forma de ácidos graxos livres circulantes através de lipólise de triacilgliceróis (Acheson *et al.*, 1980; Acheson *et al.*, 2004). Estes fatos reforçam a importância do receptor de adenosina

do tipo A1 no controle da obesidade, e portanto, deve ser considerado como um potencial alvo terapêutico para o tratamento da obesidade.

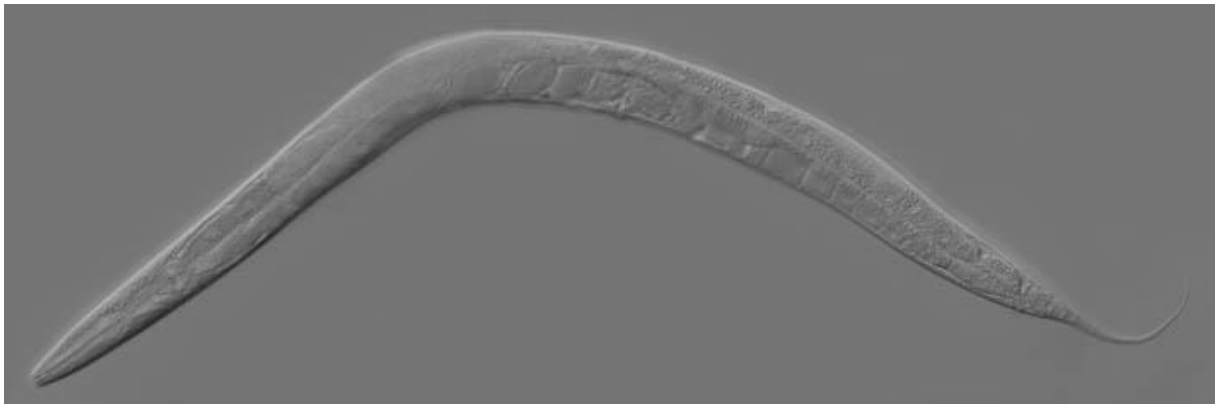
Figura 1 – Ação da Cafeína sob a fosfodiesterase (PDE)



Fonte: Baseado em http://flipper.diff.org/apptagsaccount/pathways/364

Um bom modelo para estudos relacionados ao metabolismo é *Caenorhabditis elegans* (figura 2), um nematódeo de vida livre, pequeno (±1 mm) que habita solos úmidos e usa bactérias como fonte de alimento. Em geral, se desenvolve de ovos a adultos em cerca de 2,5 dias em condições controladas de temperatura (22°C). Normalmente, após a eclosão do ovo, as larvas passam por quatro estágios larvais (L1, L2, L3 e L4) até o estágio adulto jovem e posteriormente adulto capaz de produzir ovos (Riddle *et al.*, 1997), como mostra a figura 3. Em situações extremas de temperatura ou falta de alimento, o desenvolvimento larval é interrompido no estágio L2 com formação da larva dauer, um estágio de diapausa, cujo crescimento é retomado quando se encontram novamente em um ambiente favorável (Riddle *et al.*, 1997). Devido sua transparência, técnicas não invasivas de visualização das estruturas celulares e de transcritos marcados com proteínas fluorescentes são empregadas.

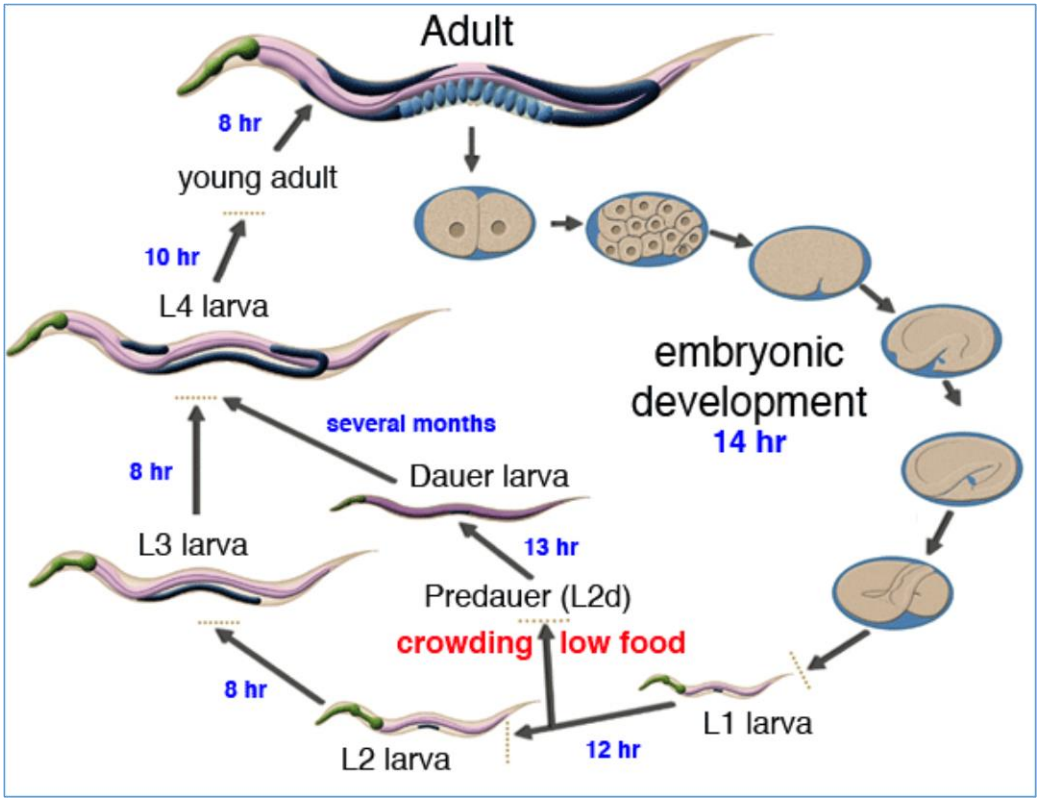
Figura 2 – *Caenorhabditis elegans* adulto



Fonte: Baseado em http://openwetware.org/wiki/Image:Adult_Caenorhabditis_elegans.jpg

O nematódeo é composto por 959 células que formam diferentes órgãos e tecidos no adulto hermafrodita (Sulston *et al.*, 1983), seu genoma é composto de aproximadamente 20 mil genes (Hodgkin, 2005), e suas vias metabólicas e biossintéticas são altamente conservados em relação aos mamíferos (Riddle *et al.*, 1997; Nass e Blakely, 2003). Pelo menos 60-80% dos genes humanos possui um ortólogo no genoma de *C. elegans* (Kaletta e Hengartner, 2006), como por exemplo o gene que codifica o receptor de adenosina, o *ador-1* (Consortium, 1998), que possibilita estudos envolvendo o sistema purinérgico no nematódeo, e o gene que codifica o receptor nuclaer hormonal, o *nhr-49*, que controla o metabolismo energético no nematódeo atravéz da expressão de enzimas da β-oxidação *(Van Gilst et al., 2005)*. Ainda, mutantes genéticos e vermes *knockouts* podem ser facilmente gerados via RNA de interferência e estão disponíveis para a pesquisa (Fire *et al.*, 1998; Wicks *et al.*, 2001), como por exemplo as cepas EG6870 *ador-1(ox489)* e STE68 *nhr-49* (nr2041) I, onde o nematódeo apresenta mutações com perda de função dos genes *ador-1* e *nhr-49*, respectivamente. Estes elementos, aliado ao fácil cultivo e manutenção, tornam *C. elegans* um atrativo modelo para estudos relacionados ao metabolismo.

Figura 3 - Ciclo de vida de *C. elegans*



Fonte: Baseado em http://www.sfu.ca/biology/faculty/hutter/hutterlab/research/Celegans.html

No nematódeo, o conteúdo lipídico é regulado pelo sistema nervoso através da modulação da alimentação e da atividade metabólica, e os triglicérides são utilizados como as principais moléculas de armazenamento energético. Muitas proteínas envolvidas em sintetizar, armazenar, oxidar e transportar lipídeos são altamente preservados entre *C. elegans* e mamíferos (Jones e Ashrafi, 2009). *C. elegans* não possui tecido de armazenamento especializado, portanto, o excesso energético é armazenado em gotículas de gordura nas células dos enterócitos, hipoderme e gônada (Ashrafi, 2007), devido sua transparência, essas reservas de gordura podem ser diretamente visualizadas nos animais intactos através da utilização de corantes específicos. A oxidação dos ácidos graxos para a produção de ATP ocorre nas mitocôndrias e peroxissomos via enzimas da β-oxidação, e resulta na formação de acetil-CoA, que serve como substrato para o ciclo do ácido tricarboxílico, e para a cadeia transportadora de elétrons, que alimentam a cadeia respiratória mitocondrial (Ashrafi, 2007).

Este estudo foi realizado considerando a relevante importância da busca de novos tratamentos para a obesidade, com baixo custo e sem efeitos colaterais, bem como o crescente consumo de compostos naturais que causam a perda de peso, este estudo foi realizado. Devido

ao grande consumo de *Ilex paraguariensis* na América do Sul (Mosimann *et al.*, 2006), em bebidas tradicionais como o chimarrão, e outros usos não tradicionais (Vieira *et al.*, 2008), como em bebidas energéticas e em chás energéticos (Bastos *et al.*, 2007), objetivamos explorar a capacidade de *Ilex paraguariensis* como um adjuvante eficaz na terapia de perda de peso *in vivo* usando *C. elegans* como um modelo de estudo.

2. OBJETIVOS

2.1 Objetivo Geral

- O presente estudo tem como objetivo investigar os efeitos do tratamento crônico com o extrato aquoso de *Ilex paraguariensis in vivo* sobre o metabolismo lipídico e comportamentos relacionados a homeostase energética, utilizando o nematódeo *Caenorhabditis elegans.*

2.2 Objetivos específicos

- Quantificar a composição do extrato aquoso de *Ilex paraguariensis* por HPLC-DAD;
- Encontrar a concentração de *Ilex paraguariensis* com atividade antioxidante frente a juglone;
- Investigar o efeito do extrato aquoso de *Ilex paraguariensis* sobre comportamentos relacionados a homeostase energética (batimentos faríngeos, duração do ciclo de defecação, frequência de curvaturas do corpo e produção de ovos)
- Analisar se o extrato aquoso de *Ilex paraguariensis* exerce alguma alteração nos níveis lipídicos em *C. elegans*.
- Avaliar se o extrato aquoso de *Ilex paraguariensis* altera o consumo de O_2 em diferentes cepas transgênicas.

3. MANUSCRITO

Ilex paraguariensis reduced fat storage in *Caenorhabditis elegans*

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Abstract

Ilex paraguariensis, is a plant widely consumed and well known for its stimulating and antioxidant properties, and could be used as modulator of fat storage in order to control obesity, a worldwide problem. Thus, the effect of chronic consumption of *I. paraguariensis* on fat metabolism was investigated using the nematode *Caenorhabditis elegans*. The animals were treated with *I. paraguariensis* at 1 mg/mL from L1-larvae-stage until adulthood. The extract concentration was chosen because exhibited antioxidant proprieties against juglone, a generator of reactive oxygen species, since these are elevated in fat tissue. Lipid accumulation, total body energy expenditure and behavioral parameters were analyzed. *Ilex paraguariensis* decreased BODIPY labeling by 63.36% compared to control without affecting behaviors related to energetic balance. Total body energy expenditure of N2, *nhr-49* and *ador-1* knockout strains was performed through oxygen consumption, and *Ilex paraguariensis* was able to increase oxygen consumption only in N2 worms. In conclusion, the synergic effects of *Ilex paraguariensis* constituents are responsible for the decreased fat deposits and increased consumption of O_2 in N2 worms dependent on NHR-49 and ADOR-1 pathways.

Keywords: fat metabolism; lipids; natural products*;* oxygen consumption; *yerba mate*.

Introduction

The prevalence of obesity is increasing worldwide, and has drawn attention of public health institutions, once is commonly associated with various metabolic disorders such as hypertension, dyslipidemia, type II diabetes, insulin resistance (Pi-Sunyer, 2004) and increased of systemic oxidative stress (Furukawa *et al.*, 2004). In 2011-2012, 34.9% of adults aged 20 years and over were obese in the United States of America (Ogden *et al.*, 2014), these data point to urgent needs for new treatments. Many methods are used to treat obesity, most of them are pharmaceuticals, which might cause collateral effects, like serious psychiatric disorders, heart attack, stroke (Kang e Park, 2012), and often, at the end of the treatment, is associated with rebound weight gain and potential drug abuse (Abdollahi e Afshar-Imani, 2003). Thus, natural compounds with antioxidant proprieties could be good candidates for new anti-obesity treatments, once they can cause less adverse effects and can be easily added to the diet (Hasani-Ranjbar *et al.*, 2009).

A diversity of plants are used as a complementary or alternative approaches in regular diet worldwide (Debas *et al.*, 2006). *Ilex paraguariensis* St. Hil. Var. *paraguariensis* (Aquifoliaceae) is widely used in southern Brazil, northern Argentina, Paraguay and Uruguay (Mosimann *et al.*, 2006) as a drink called *chimarrão, tererê, or mate*. Their consumption has been popular for centuries because its stimulant and medicinal properties (Bracesco *et al.*, 2011). Effects of *Ilex paraguariensis* consumption include: the central nervous system stimulation (Gonzalez *et al.*, 1993), thermogenic (Arcari *et al.*, 2009), stimulating properties *in vitro* (Filip *et al.*, 2000) and increase antioxidant defenses (Schinella *et al.*, 2000).

Previous studies reported that the main compound found on aqueous extract of *Ilex paraguariensis* were methylxanthines, mainly caffeine (Filip *et al.*, 1998; Filip, R *et al.*, 2001). Caffeine is well-known as a noncaloric thermogenic agent, related to metabolic rates increase (Higgins e Means, 1915; Haldi *et al.*, 1941; Miller *et al.*, 1974), fat oxidation

induction (Dulloo *et al.*, 1989; Astrup *et al.*, 1990; Bracco *et al.*, 1995), respiratory centers stimulation (Haldi *et al.*, 1941) and increase the resting energy expenditure (Belza *et al.*, 2009). The caffeine mechanism of action appears to be the antagonism of adenosine receptors, even though caffeine also inhibits phosphodiesterases (Jacobson, 2009), and this results in a consistent reduction of the lipid depots.

Animal models are useful tools to evaluate the efficacy of compounds in the prevention and treatment of obesity (Kang *et al.*, 2012). *Caenorhabditis elegans*, an alternative animal model, was used here to better understand lipid profile *in vivo* after a chronic treatment with *Ilex paraguariensis*. This nematode has been described as an emergent system to study a variety of biological processes, including fat metabolism. Once many of the proteins involved in synthesizing, oxidation and transport of lipids are highly conserved between *C. elegans* and mammals (Jones e Ashrafi, 2009), some new key pathways could be studied through this model. Fat oxidation is broadly associated with respiratory activity, which can be easily measured by oxygen rate consumption (Shoyama *et al.*, 2009).

Considering the widely consumption of *Ilex paraguariensis* (Rosovsky, 1983), the crescent interest in natural compounds which stimulate metabolism and weight loss and that obesity is a world health problem, we studied *Ilex paraguariensis*, a plant with a mix of bioactive constituents, in order to explore its modulation of fatty acid metabolism *in vivo* using *C. elegans* as animal model. In this way, our work aimed to describe the pathways involved in the modulation of fat metabolism by *Ilex paraguariensis* and the possibility to use this plant as an adjuvant in obesity treatment to control weight gain.

Materials and methods

Chemical, apparatus and general procedures of analytical grade

All chemical were of analytical grade. Methanol, formic acid, gallic acid, chlorogenic acid and caffeic acid were purchased from Merck (Darmstadt, Germany). Quercetin, theobromine, caffeine, rutin, catechin, epigallocatechin and kaempferol were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

Plant material and extract preparation

Minced leaves of *Ilex paraguariensis* used in this study were purchased in local markets. The extraction was carried out by pouring 100 mL of boiled distilled water on plant sample (Gorjanovic *et al.*, 2012). After extraction at room temperature (10 min), the infusion was filtered using a sterilization filter with 0.22μm pore size.

Quantification of compounds by HPLC-DAD

Reverse phase chromatographic analyses were carried out under gradient conditions using C18 column (4.6 mm x 250 mm) packed with 5μm diameter particles. The mobile phase was water containing 1% formic acid (A) and methanol (B), and the composition gradient was: 15% of B until 10 min and changed to obtain 20%, 30%, 50%, 60%, 70%, 20% and 10% B at 20, 30, 40, 50, 60, 70 and 80 min, respectively, following the method described by Abbas

et al. (2014) with slight modifications (Abbas *et al.*, 2014). *Ilex paraguariensis* infusion was analyzed at a concentration of 20 mg/mL. The presence of ten antioxidant compounds was investigated, namely, gallic acid, chlorogenic acid, caffeic acid, catechin, epigallocatechin, quercetin, rutin, kaempferol, caffeine and theobromine. Identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.7 ml/min, injection volume 40 µl and the wavelength were 257 nm for gallic acid, 270 nm for theobromine, 280 nm for catechin, epigallocatechin and caffeine, 327 nm for caffeic and chlorogenic acids, and 366 nm for quercetin, rutin and kaempferol. All the samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.030 – 0.250 mg/ml for kaempferol, quercetin, catechin, epigallocatechin, rutin, caffeine and theobromine; and 0.045 – 0.300 mg/ml for gallic, caffeic and chlorogenic acids. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 600 nm). Calibration curve for gallic acid: $Y = 13057x + 1285.4$ ($r = 0.9998$); catechin: $Y = 12728x + 1197.5$ ($r = 0.9995$); epigallocatechin: $Y = 11893 + 1357.2$ ($r = 0.9995$); chlorogenic acid: $Y = 12659x + 1287.8$ ($r = 0.9993$); caffeic acid: $Y = 11962x + 1326.2$ ($r = 0.9997$); caffeine: $Y = 13276x + 1297.6$ ($r = 0.9999$); theobromine: $Y = 12473x + 1175.8$ ($r = 0.9996$); rutin: $Y = 13805 + 1195.7$ ($r = 0.9999$); quercetin: $Y = 13627x + 1362.1$ ($r = 0.9999$) and kaempferol: $Y = 12583x + 1274.8$ ($r = 0.9997$). All chromatography operations were carried out at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 σ/S, respectively, where σ is

the standard deviation of the response and S is the slope of the calibration curve (Boligon *et al.*, 2013).

C. elegans strains, growth conditions and *Ilex paraguariensis* treatment

Wild-type *C. elegans* strain N2 (var. Bristol) and STE68 *nhr-49* (nr2041) I. were provided by the *Caenorhabditis* Genetics Center (University of Minnesota, USA). Nuclear hormone receptor NHR-49, a mammalian HNF4 homolog, is a key regulator of fat usage, transcriptionally regulates many rate-limiting genes of beta-oxidation, including acyl-CoA synthetase, enoyl-CoA hydratase and carnitine palmitoyl transferase (Van Gilst *et al.*, 2005). EG6870 strain, *ador-1(ox489),* was kindly supplied from Dr. Erik Jorgensen laboratory (University of Utah, USA). This strain has a deletion from 1kb upstream and the first three exons of the *ador-1* gene, and was outocrossed six times. *Ador-1* gene encodes an ortholog of human adenosine receptor (Consortium, 1998). All strains were maintained at 20ºC.

Treatment plates were prepared diluting *I. paraguariensis* extract in distilled autoclaved water and spreading it with *Escherichia coli* OP50 to the surface of dry nematode grow media (NGM) agar plates (Brenner, 1974) to final concentrations used. Control plates were prepared with water and bacteria at the same proportions. Plates were incubated overnight at 37°C to allow bacteria growth.

To synchronize worms, embryos were obtained from gravid adult hermaphrodites by bleach solution containing 1% NaClO and 0.1 M NaOH (Sulston e Hodgkin, 1988). Eggs were allowed to hatch overnight in M9 buffer (42 mM Na_2HPO_4, 22 mM KH_2PO_4, 8.6 mM NaCl and 1 mM $MgSO_4$), then synchronized L1 worms were cultured onto plates in the presence or absence of extract and allowed to develop until the 1-day-adult stage at 20°C. In

all treatments, the worms were previously collected, washed at least three times with M9 buffer, and then the experiments were carried out.

Assessment of a concentration resistant to oxidative stress

After treatment with *Ilex paraguariensis* at 0,25, 0,5 and 1 mg/mL, the 1-day-old N2 wild-type nematodes were exposed to 100 uM of juglone, also called 5-hydroxy-1,4-naphthoquinone (IUPAC), a generator of reactive oxygen species (ROS) (Blum e Fridovich, 1983), this concentration is supposed to kill approximately 50% of the nematodes (LD50) (Arantes *et al.*, 2014). Juglone was prepared in EtOH (1% final concentration). After 1 hour at 20°C, 100 nematodes per treatment were assessed with a Nikon E200 microscope (Tokyo, Japan). Animals that reacted to a mechanical stimulus were scored as alive, and non-responding animals were considered to be dead. Analyses were carried out in five independent assays. Results are shown as percentage of control.

Bacterial Growth Curve

E. coli OP50 growth was evaluated over 4 h in the presence or absence of 1mg/mL of *Ilex paraguariensis*. Growth curves were measured on a spectrophotometer by determining the optical density at 600 nm, and were normalized with the control group at time zero (Bonomo Lde *et al.*, 2014). The experiments were performed in three independent assays.

C1-BODIPY-C12 staining

C1-BODIPY-C12 conjugated fatty acids (BODIPY) lipid staining were carried out as previously described (Mak *et al.*, 2006). C1-BODIPY-C12 was dissolved in DMSO, and a 5

mM stock solution was stored at -20ºC. Dye stock solutions were freshly diluted to 1 µM, and 0.5 mL was applied to the surface of NGM plates (10 mL agar) seeded with *E. coli* OP50 and 0 or 1 mg/mL of *Ilex paraguariensis* to a 50 nM final concentration. Plates were allowed to dry in a laminar flow hood and were immediately used. Synchronized wild-type L1-stage worms were transferred to these plates and allowed to develop until adulthood. One-day-adults were mounted on agar pads and immobilized with 10 mM sodium azide for image acquisition using identical settings and appropriate filters with a Zeiss Axiovert II microscope (Thornwood, NY, USA) fitted with a CCD camera. Fluorescence of the two first intestinal pairs of cells was used for quantification and analyses of images were conducted with ImageJ software. The experiments were performed in three independent assays with twenty worms per group.

Oxygen consumption

Oxygen consumption rate in wild-type worms was measured with a Hansatech Oxymeter (Pentney, Norfolk, UK) with a Clark-type electrode. The electrode chamber was washed and stabilized for 30 min with 1 mL air-saturated M9 buffer before analysis. One-day-old worms from control and *I. paraguariensis* treatment were washed 5 times with M9 buffer to remove residual bacteria. Around 2,000 worms were transferred to a cuvette with 1 mL of M9, and oxygen consumption was measured for 2–15 min at 20ºC to obtain oxygen consumption rates (Ranjan *et al.*, 2013). The experiments were performed in five independent assays.

Pharynx pumping rate

Pharyngeal bulb contractions were measured at 20 ± 2ºC in 1-day-old N2 wild-type worms on their treatment plates. The number of pharynx pumps in a 10s-interval, in triplicate (Huang *et al.*, 2004), was assessed with a Nikon E200 microscope (Tokyo, Japan). Analyses were carried out in three independent assays with ten worms per group. Results are shown as pharynx pumping/minute.

Defecation assay

Defecation frequencies were performed by observing 1-day-old N2 wild-type worms in their plates of treatment (Migliori *et al.*, 2011) with a Nikon E200 microscope (Tokyo, Japan). The defecation cycle length was defined as the duration between the pBoc steps (posterior body muscle contraction) of two consecutive defecations. Analyses were carried out in three independent assays with ten worms per group.

Body bends frequency

After treatment, 1-day-old N2 wild-type worms were randomly transferred to food-free NGM plates and allowed to freely move for 3 min to adaptation. The number of times each worm changes the direction of the body was scored with a Nikon E200 microscope (Tokyo, Japan) during a 20s-interval in triplicate (Tsalik e Hobert, 2003). Analyses were carried out in three independent assays with ten worms per group.

Egg-production

To assess the number of eggs inside the uterus, 1-day-old N2 wild-type nematodes from control and treatment plates were individually picked into a drop of bleaching solution (1% NaOCl, 0.25 M NaOH). The worms' cuticles were disrupted and released eggs were counted (Schafer e Kenyon, 1995) with a Nikon E200 microscope (Tokyo, Japan). Analyses were carried out in three independent assays with ten worms per group

Statistical analyses

Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA). The statistical differences between conditions were determined by a student's t-test, while the statistical differences between different concentrations of *Ilex paraguariensis* were determined by a one-way ANOVA followed by Bonferroni's *post-hoc* test. Values are represented as means ± SEM, results were considered statistically significant when $p < 0.05$.

Results

HPLC analysis of Ilex paraguariensis bioactive compounds

HPLC fingerprinting of *Ilex paraguariensis* infusion revealed the presence of the gallic acid (t_R = 9.97 min; peak 1), catechin (t_R = 15.03 min; peak 2); chlorogenic acid (t_R = 21.38 min; peak 3), caffeic acid (t_R = 24.19 min; peak 4), caffeine (t_R = 27.48 min; peak 5), theobromine (t_R = 32.65 min; peak 6), epigallocatechin (t_R = 35.11 min; peak 7), rutin (t_R = 39.45 min; peak 8), quercetin (t_R = 49.27 min; peak 9) and kaempferol (t_R = 54.67 min; peak 10) (Table 1).

Ilex paraguariensis on resistant to oxidative stress

Since fat accumulation is correlated with systemic oxidative stress in humans and mice (Furukawa *et al.*, 2004), was determined a concentration of *Ilex paraguariensis* capable to reduce the oxidative damage caused by juglone, a generator of ROS (Blum e Fridovich, 1983). The 1-hour exposure of 1-day-old N2 wild-type nematodes to 100 mM of juglone killed approximately 45,16% of worms, and the pretreatment with 1mg/mL of *Ilex paraguariensis* increased nematode survival after juglone exposure by 22.12% (Fig. 1, $p<0.001$). No differences were observed between 0.25 and 0.5 mg/mL of extract and control group.

Antimicrobial effect of *Ilex paraguariensis*

Once killed bacterial can provide differences in micronutrients and be responsible for some difference in the range of fat stores in *C. elegans* (Brooks *et al.*, 2009; Yilmaz e Walhout, 2014), antimicrobial activity of *Ilex paraguariensis* on *E. coli* OP50 was tested. No differences were observed between bacterial growth in the presence or absence of 1 mg/mL of *Ilex paraguariensis* (data not shown).

Effects of *Ilex paraguariensis* on *C. elegans* fat storage

BODIPY-conjugated fatty acids stain was used to evaluate changes in fat depots in adult worms after *Ilex paraguariensis* treatment. A reduction of 37,57% in fluorescence levels was observed in worms exposed to 1mg/mL *I. paraguariensis* compared to control group (Fig. 2, $p<0.0001$). These data suggest that exposure to the extract decreased fat storage.

Behavior parameters in *Ilex paraguariensis* treated worms

Treatment with *Ilex paraguariensis* did not affect pharynx pumping rate and defecation cycle length of 1-day-old adult wild-type worms. Both parameters were similar to controls in the presence of 1 mg/mL of extract (Fig. 3A, B). Movement rate was assessed through frequency of body bends. Our results show that treatment did not change locomotion behavior in 1-day-old *C. elegans* treated (Fig. 3C). Finally, egg production was also assessed in 1-day-old adult worms after extract treatment and there were no differences between control and treated worms (Fig. 3D).

Oxygen consumption in wild-type *C. elegans*

The oxygen consumption rate was compared between untreated and *Ilex paraguariensis* treated nematodes from wild-type (N2), STE68 nhr-49(nr2041) I. and EG6870 *ador-1(ox489)* strains, as a measure of total body energy expenditure. The oxygen consumption rate in N2 treated animals was increased by 70,16% in relation to control animals ($p<0.05$; Fig. 4A). STE68 nhr-49(nr2041) I. and the EG6870 ador-1(ox489) treated worms did not showed significative differences from control ($p<0.05$; Fig. 4B, C)

Discussion

The concentration of *I. paraguariensis* used here was chosen based on a concentration that did not present antimicrobial activity, and consequently did not supply differences in micronutrients that could affect the range of lipid content in treated *C. elegans* (Brooks *et al.*, 2009; Yilmaz e Walhout, 2014). Besides the oral food intake, animals also absorb compounds through cuticle, a protective layer of specialized extracellular matrix, consisting primarily of

collagen, lipids, and glycoproteins, which is a highly impermeable barrier between the animal and environment (Page e Johnstone, 2007) making necessary the use of high concentrations of extracts/products/compounds in experimental protocols.

Because increased oxidative stress in accumulated fat is an important pathogenic mechanism of obesity (Keaney *et al.*, 2003; Furukawa *et al.*, 2004), we decided choose a concentration resistant to oxidative stress that could help in the prevention or improve the treatment of obesity. Was tested three concentrations of *Ilex paraguariensis*, and the best one that exhibited properties of an antioxidant against the juglone was 1mg/mL of the extract. Based on this found, we provide all of this work with this concentration. Hereafter, we assessed fat depots through BODIPY-conjugated fatty acids stain, and was founded that chronic treatment with 1mg/mL of *Ilex paraguariensis* was able to reduce the *C. elegans* fat storage in 1-day-old N2 wild-type.

This study aimed to understand lipid profile after a chronic treatment with *Ilex paraguariensis* in a whole organism, since this plant is known to be a natural thermogenic and stimulant. Survival, growth and reproduction in animals depend on preservation of appropriate energy reservoirs (Wallace, 2010), in worms, fat storage represents the balance between energy intake and expenditure. Thereby, we speculate if the decrease of fat depots induced by *I. paraguariensis* could be caused by a reduction in food intake. Our results indicate that decrease in fat storage, induced by extract, was not related to caloric restriction or to an increase in defecation rate. Alternatively, body movements are also associated to alterations in lipid deposits, once enhancement in movement parameters could increase the use of energy reserves, leading to a decrease in fat storage (Watts *et al.*, 2003). However, our results show that the decline of lipid storage induced by *I. paraguariensis* treatment was not caused by some modification in body movements, or a disruption in egg production can cause some consequence on store of lipid droplets. In oocytes production, yolk, polyunsaturated

fatty acids are transported from the site of fat metabolism, the intestine (Kubagawa *et al.*, 2006). Thus, an increase in egg production can indirectly alter the fat metabolism. In this study, *I. paraguariensis* did not alter egg production.

Considering the peripheral mechanisms of thermogenesis, total body expenditure was measured through oxygen consumption. Our results demonstrate that *I. paraguariensis* is able to increase the oxygen consumption of worms in our protocol experiment. As a consequence, this increase in O_2 consumption can be associated to an increase in beta-oxidation, once it can decrease the fat storage. Therefore, the involvement of fatty acid oxidation in the lipid-reducing effects of *I. paraguariensis* was investigated in *C. elegans* through a key regulator gene of fat oxidation, the nuclear hormone receptor, NHR-49 (Chamoli *et al.*, 2014).

The NHR-49 regulates fatty acid oxidation and lead to depletion of stored fat in *C. elegans* (Liang *et al.*, 2010). Our results demonstrate that *Ilex paraguariensis* was unable to increase the consumption of O_2 in *nhr-49* knockout worms. Once NHR-49 targets multiple enzymes involved in β-oxidation (Van Gilst *et al.*, 2005), *I. paraguariensis* effects in fat storage could be due to beta-oxidation pathway through *nhr-49* gene. Thus, we supposed that the increase in beta-oxidation pathway produces an increase in the availability of acetyl-CoA to the mitochondrial tricarboxylic acid (TCA) cycle (Lodish *et al.*, 2000), that feeds the electron transport chain, which increases O_2 consumption in the respiratory chain.

Ilex paraguariensis has been related to various biological activities, which have been mainly attributed to its large amount of bioactive compounds, as the methylxanthines caffeine and theobromine, phenolic compounds such as caffeic acid, chlorogenic acid, and saponins (Bastos *et al.*, 2007). The benefic effects exercised by *Ilex paraguariensis* on adipose tissue can be lost when specific compounds are isolated, the same effect could be exerted by isolated compounds only in high concentrations, which indicates that some of these properties may be related to the synergistic effects of some compounds (Carlini, 2003). Taking this, the most

abundant compounds present in this extract are caffeic acid, which has antioxidant proprieties (Gulcin, 2006), and caffeine, which has the potential to produce significant effects such as metabolism stimulation (Westerterp-Plantenga *et al.*, 2006), what could make this extract a good adjuvant in obesity treatment.

Caffeine is an adenosine A1 receptor antagonist. The A1 receptors are coupled to inhibitory GTP binding proteins (G proteins), blocking the adenylate cyclase enzyme activation (Linden, 1991). This way, the binding of adenosine to the A1 receptor leads to reduction of cyclic AMP levels (cAMP) in adipose tissue, and thus inhibits lipolysis (Challiss *et al.*, 1992). The binding of caffeine to this receptor increases the cAMP production and consequently increases the lipolysis in adipocyte tissue (Nehlig *et al.*, 1992). Another mechanism of caffeine is the inhibition of phosphodiesterase (PDA), and thus, reduces the intracellular breakdown of cyclic AMP (cAMP), resulting in an increase in the concentration of cAMP and a subsequent increase in lipolysis (Nehlig *et al.*, 1992).

Therefore, the purinergic system could be, in some way, involved in the mechanism of action affecting the lipid metabolism in *C. elegans*. To confirm our hypothesis the oxygen consumption of *ador-1* knockout, which encodes an ortholog of human adenosine receptor, were performed. As similar to NHR-49, *Ilex paraguariensis* did not change the O_2 consumption of *ador-1* knockout strain. Considering this, *Ilex paraguariensis* could reduce lipid storage in *Caenorhabditis elegans* acting through purinergic system.

Conclusion

In conclusion, for the first time, at the best of our knowledge, our study demonstrated that chronic treatment with *Ilex paraguariensis* was able to decrease the deposits of lipids in 1-day-old wild-type *Caenorhabditis elegans*. This effect could be, at least in part, caused by an increase on beta-oxidation pathway, through the *nhr-49* gene, inducing an increase in O_2

consumption in the respiratory chain, as a result of an increase in TCA cycle products, independently of alterations in behaviors related to energy expenditure. In addition, the involvement of purinergic system in the lipid metabolism of *C. elegans* was demonstrated, suggesting that adenosine receptors could be a target in future studies of lose weight, in the control of obesity or in abnormalities of fat storage.

The extract of *Ilex paraguariensis* might cause a reduction in fat storage in N2 wild-type *C. elegans* through a synergic effect of its bioactive constituents. This effect could be exerted by isolated compounds only in high concentrations, which might be toxic. So, due its game of bioactive compounds, *Ilex paraguariensis* could be used as an adjuvant in therapies of weight loss.

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Conflict of Interest statement

The authors declare no conflicts of interests.

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Figure captions

Figure 1. *Ilex paraguariensis* increased the worm survival after 1-hour exposure to juglone 100 µM in *Caenorhabditis elegans* wild-type (N2). Survival of 1-day-old adults N2 wild-type exposed to 100 µM juglone during 1 h. Data are expressed as percentage of living worms. **p<0.001, statistically different compared to untreated group by one-way ANOVA followed by Bonferroni Multiple Comparison Test (mean, SEM, *n*= approximately 100 worms per group). The experiment was performed five times at different days.

Figure 2. Fluorescence decrease induced by *Ilex paraguariensis* exposition in *Caenorhabditis elegans* wild-type (N2). (A) Visualization of lipid droplets evidenced by BODIPY labeling and (B) fluorescence quantification in 1-day-old adults N2 wild-type. ***p<0.001, statistically different compared to untreated group by Student's T-test (mean, SEM, *n* =60). The experiments were performed three times at different days.

Figure 3. *Caenorhabditis elegans* wild-type behavior after *Ilex paraguariensis* treatment. Effect of *I. paraguariensis* on (A) pharyngeal pumping rate, (B) defecation cycle length, (C) body bends and (D) egg production in 1-day-old adults N2 wild-type. No statistic difference was found compared to untreated group (mean, SEM, *n* =30). The experiments were performed three times at different days.

Figure 4. Measurement of oxygen consumption rate on *Caenorhabditis elegans* treated with *Ilex paraguariensis.* Oxygen consumption rates in 1-day-old adults worms. (A) Wild type, (B) STE68 nhr-49(nr2041) I. and (C) EG6870 ador-1(ox489). **p<0.01, statistically different compared to untreated group by Student's T-test (mean, SEM, *n* =7). The experiments were performed 5 times at different days.

Table 1 – Composition of *Ilex paraguariensis* infusion.

Compounds	*Ilex paraguariensis*		LOD	LOQ
	mg/g	%	μg/mL	μg/mL
Gallic acid	1.27 ± 0.01 a	0.12	0.015	0.049
Catechin	2.98 ± 0.03 b	0.29	0.032	0.105
Chlorogenic acid	3.71 ± 0.01 b	0.37	0.008	0.027
Caffeic acid	9.15 ± 0.02 c	0.91	0.021	0.070
Caffeine	8.86 ± 0.01 d	0.88	0.029	0.095
Theobromine	3.65 ± 0.01 b	0.36	0.007	0.023
Epigallocatechin	6.01 ± 0.03 e	0.60	0.016	0.052
Rutin	7.43 ± 0.02 f	0.74	0.026	0.086
Quercetin	3.12 ± 0.01 b	0.31	0.035	0.115
Kaempferol	5.95 ± 0.03 e	0.59	0.019	0.063

Results are expressed as mean ± standard deviations (SD) of three determinations.

Averages followed by different letters differ by Tukey test at $p < 0.05$.

Figure 1

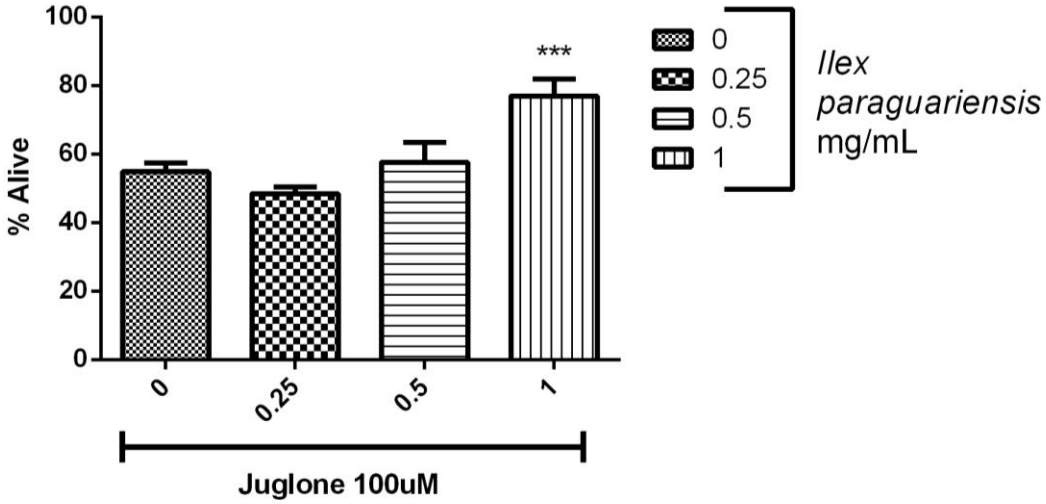


Figure 2

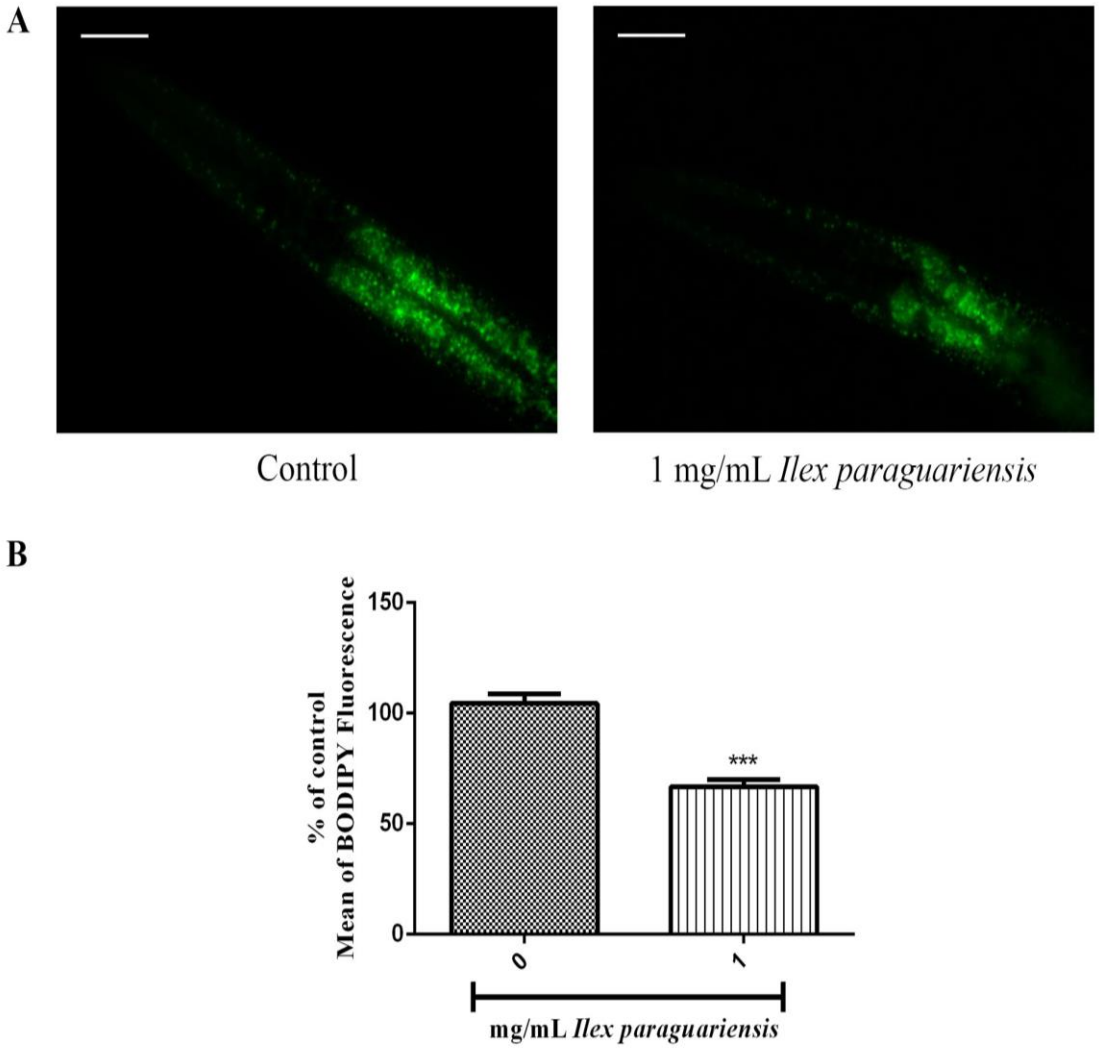

A
Control
1 mg/mL *Ilex paraguariensis*
B
% of control
Mean of BODIPY Fluorescence
150
100
50
0
***
0
1
mg/mL *Ilex paraguariensis*

Figure 3

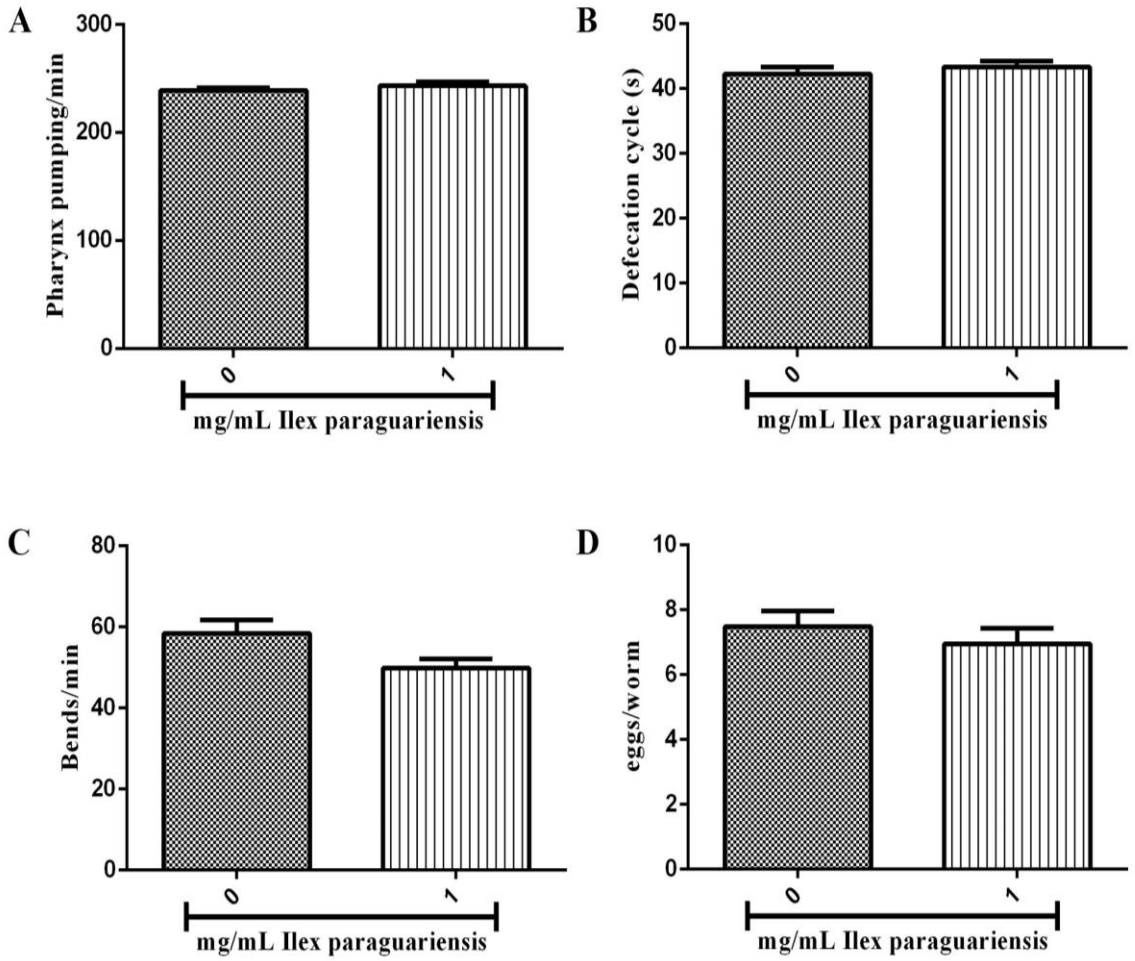
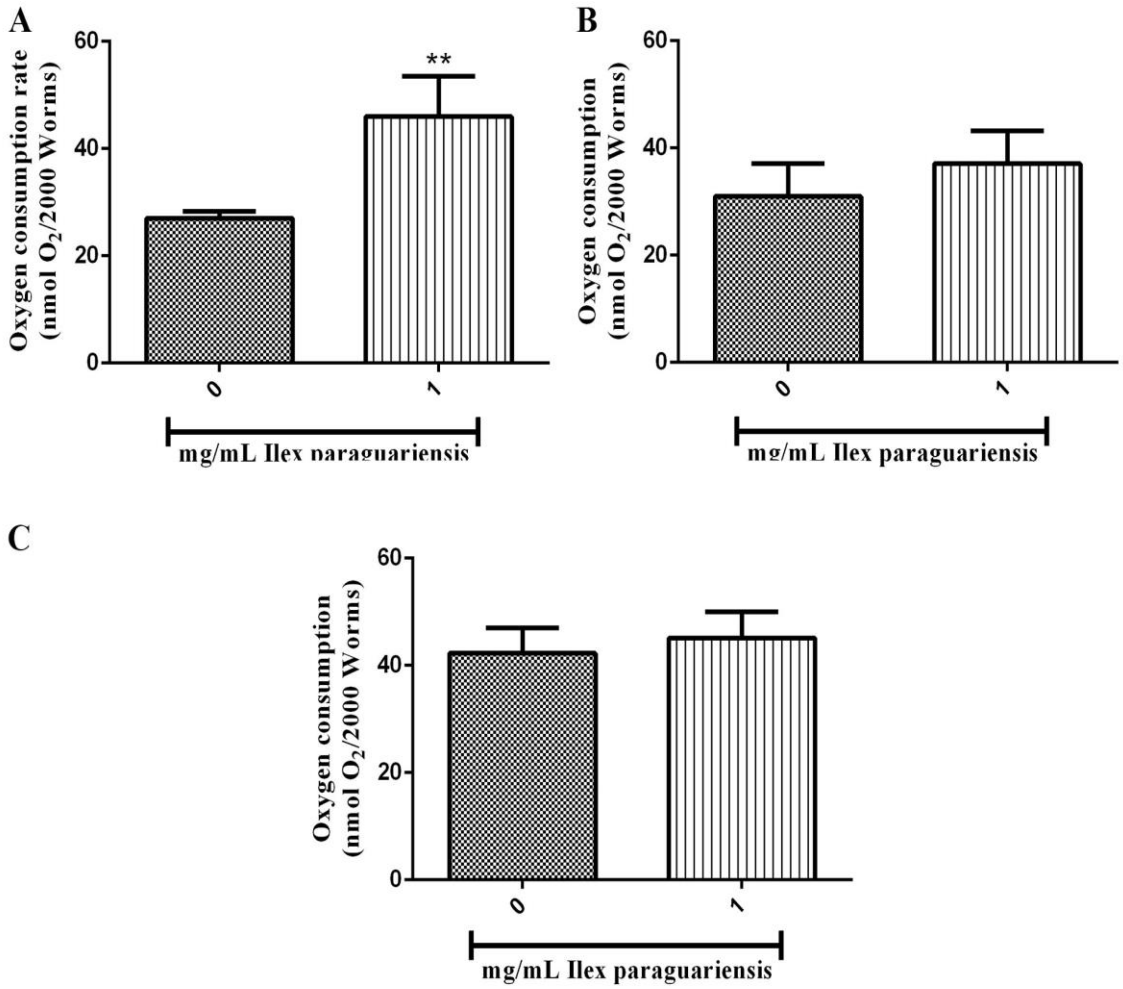

A
Pharynx pumping/min
300
200
100
0
0
1
mg/mL Ilex paraguariensis
B
Defecation cycle (s)
50
40
30
20
10
0
0
1
mg/mL Ilex paraguariensis
C
Bends/min
80
60
40
20
0
0
1
mg/mL Ilex paraguariensis
D
eggs/worm
10
8
6
4
2
0
0
1
mg/mL Ilex paraguariensis

Figure 4


A
Oxygen consumption rate (nmol O_2/2000 Worms)
60
40
20
0
**
0
1
mg/mL Ilex paraguariensis
B
Oxygen consumption (nmol O_2/2000 Worms)
60
40
20
0
0
1
mg/mL Ilex paraguariensis
C
Oxygen consumption (nmol O_2/2000 Worms)
60
40
20
0
0
1
mg/mL Ilex paraguariensis

4. CONCLUSÕES

Os resultados mostrados neste trabalho elucidam o potencial farmacológico da espécie vegetal *Ilex paraguariensis* para tratamento e controle da obesidade. Neste estudo, foi demonstrado pela primeira vez que o tratamento crônico com o extrato aquoso de *Ilex paraguariensis* foi eficaz em reduzir os depósitos de lipídeos no nematódeo *Caenorhabditis elegans* de 1 dia de vida adulta.

Através de análises comportamentais, foi observado que os principais fenótipos relacionados ao gasto energético não foram alterados por *Ilex paraguariensis* na concentração testada, portanto, um aumento da demanda energético não pode explicar as modificações observadas nos depósitos de lipídeos. Além disso, a concentração usada de *Ilex paraguariensis* não apresentou atividade antimicrobiana, portanto, não fornece diferenças de micronutrientes que poderiam afetar o teor de lipídeos em *C. elegans* tratados.

Devido à grande quantidade de compostos presente no extrato aquoso de *Ilex paraguariensis*, torna-se difícil precisar qual o mecanismo exato pelo que a planta atua para reduzir os depósitos de lipídeos em *C. elegans*, mas acreditamos que a interação entre os constituintes da planta gera um efeito sinérgico capaz de ativar a via da β-oxidação e o aumento da lipólise para reduzir os depósitos de lipídeos.

Sugerimos, que os receptores de adenosina podem ser um alvo para futuros estudos relacionados a obesidade, e que *Ilex paraguariensis* pode ser um bom adjuvante no controle e tratamento da obesidade. Por fim, todos estes indícios deixam claro que *C. elegans* é um modelo para estudos preliminares relacionados ao controle e tratamento da obesidade, visto que estudos mais aprofundados são necessários.

5. PERSPECTIVAS

A partir dos resultados obtidos, mais estudos são necessários para identificar precisamente o mecanismo de ação de *Ilex paraguariensis* para diminuir os depósitos de lipídeos em *C. elegans.*

Nossos estudos têm se direcionado para a pesquisa dos efeitos da cafeína isolada sob os depósitos de lipídeos de *C. elegans*, e também analisar outras plantas popularmente usadas e com grande quantidade de cafeína, como por exemplo, *Paullinia cupana*, o guaraná.

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