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EFEITOS DO CONSUMO DE SACAROSE SOBRE PARÂMETROS METABÓLICOS, DESENVOLVIMENTAIS E ANTIOXIDANTES EM *Drosophila melanogaster:* PAPEL DAS PLANTAS *Syzygium cumini* e *Bauhinia forficata*

DISSERTAÇÃO DE MESTRADO

Assis Ecker

Santa Maria, RS, Brasil

2014

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Assis Ecker

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

Orientador: Prof. Dra. Nilda Vargas Barbosa

Santa Maria, RS, Brasil

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Mestre em Bioquímica Toxicológica

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Enfim, agradeço a todos que contribuíram de alguma forma para que eu chegasse até aqui,

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APRESENTAÇÃO

No item **INTRODUÇÃO**, está descrita uma revisão sucinta sobre os temas trabalhados nesta dissertação.

Os resultados que fazem parte desta dissertação bem como a metodologia empregada estão apresentados sob a forma de um manuscrito redigido em inglês conforme as normas do periódico ao qual foi submetido para publicação. Os itens Introdução, Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas, encontram-se nos próprio artigo e representam a íntegra deste estudo.

Os itens **CONCLUSÃO** e **PERSPECTIVAS** encontram-se no final desta dissertação, e apresentam as conclusões gerais sobre os resultados do manuscrito e as perspectivas para trabalhos futuros.

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

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

EFEITOS DO CONSUMO DE SACAROSE SOBRE PARÂMETROS METABÓLICOS, DESENVOLVIMENTAIS E ANTIOXIDANTES EM *Drosophila melanogaster*: PAPEL DAS PLANTAS *Syzygium cumini* e *Bauhinia forficata*

AUTOR: ASSIS ECKER
ORIENTADORA: NILDA VARGAS BARBOSA
Local e Data da Defesa: Santa Maria, 14 de Março de 2014.

A mosca da fruta, *Drosophila melanogaster*, tem sido considerada um organismo modelo adequado para a investigação de disfunções metabólicas/desenvolvimentais e estratégias terapêuticas. Neste trabalho foram utilizadas larvas de *Drosophila melanogaster* para avaliar o papel das plantas *Syzygium cumini* e *Bauhinia forficata* sobre os efeitos provocados pelo consumo de dietas ricas em sacarose como marcadores de estresse oxidativo e respostas fenotípicas associadas à sinalização da insulina. Os experimentos foram realizados com larvas do primeiro estágio (L1), coletadas 24 horas após a deposição dos ovos. As larvas foram tratadas com dietas ricas em sacarose (15 e 30%) suplementadas ou não com 5mg/mL dos extratos aquosos de *S. cumini* e *B. forficata*. As moscas recém-eclodidas das larvas (1-3 dias de idade) foram utilizadas para avaliar os parâmetros bioquímicos. Durante o estágio larval, o consumo das dietas ricas em sacarose 15 e 30% atrasou o tempo para pupação e reduziu o número de pupas brancas. As moscas nascidas de larvas tratadas com sacarose 30% também tiveram uma diminuição significativa do peso corporal quando comparado com as moscas do grupo controle. O consumo de ambas as dietas elevou os níveis de glicose+trealose na hemolinfa e de triglicerídeos na hemolinfa e em homogenato do corpo total nas moscas adultas. Os níveis de H_20_2 foram aumentados no homogenato das moscas nascidas de larvas que cresceram em ambas as dietas quando comparados ao controle; no entanto somente a dieta com sacarose 30% induziu perda da viabilidade mitocondrial. A ingestão desta dieta também causou uma diminuição na atividade das enzimas superóxido dismutase, glutationa S-transferase, acetilcolinesterase e δ-aminolevulinato desidratase, bem como um aumento na atividade da catalase em moscas adultas. A suplementação com os extratos de *S. cumini* e *B. forficata* reverteu a maioria das disfunções metabólicas e desenvolvimentais provocadas pelas dietas ricas em sacarose. No entanto, o extrato de *S. cumini* foi mais eficiente que a *B forficata* em reduzir a hiperglicemia promovida pela ingestão de ambas as dietas e as alterações no status antioxidante causadas pela dieta rica em sacarose 30%. No geral, os resultados obtidos destacam a *D. melanogaster* como um organismo modelo efetivo para investigar condições que alteram a homeostase metabólica e apontam principalmente a planta *S. cumini* como agente promissor para estudos relacionados com desordens metabólicas ligadas ao excesso de açúcar.

Palavras chave: Drosophila melanogaster, Syzygium cumini, Bauhinia forficata, Sacarose, Estresse Oxidativo

ABSTRACT

Master's Degree Dissertation
Graduation Program in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria, RS, Brazil

EFFECTS OF SUCROSE CONSUMPTION ON METABOLIC, DEVELOPMENTAL AND ANTIOXIDANT PARAMETERS IN *Drosophila melanogaster:* ROLE OF *Syzygium cumini* and *Bauhinia forficata* PLANTS

AUTHOR: ASSIS ECKER
ADVISOR: NILDA VARGAS BARBOSA
Place and Date of the Defense: Santa Maria, March 14th, 2014

The fruit fly, *Drosophila melanogaster*, has been considered a suitable model organism for investigation of developmental/metabolic dysfunctions and therapeutic strategies. In this work *Drosophila melanogaster* larvae were used to evaluate the role of the plants *Syzygium cumini* and *Bauhinia forficata* on the effects triggered by consumption of high-sucrose diets (HSD) as oxidative stress markers and phenotypic responses associated to insulin signaling. The experiments were performed with first instar larvae (L1), collected 24 hr after egg deposition. The larvae were fed on high-sucrose diets (15 and 30%) supplied or not with 5mg/mL of *S. cumini* and *B. forficata* aqueous extracts. Newly-eclosed flies from larvae (1-3 day-old) were used to assess biochemical parameters. During the larval stage, 15% HSD and 30% HSD intake delayed the time to pupation and reduced the number of white pupa. The flies hatched from larvae treated with 30% sucrose also had a significant decrease in body weight compared with the flies from control group. The consumption of both diets increased the hemolymph glucose+treahalose levels and hemolymph/whole body homogenate triglycerides in adult flies. H_20_2 levels were also increased in the homogenate of flies hatched from larvae grown on both diets when compared to control; however only 30% sucrose diet induced loss of mitochondrial viability. The intake of this diet also caused a decrease in the activity of superoxide dismutase, glutathione S-transferase, acetylcholinesterase and δ-D-aminolevulinate dehydratase enzymes as well as an increase in catalase activity in adult flies. Supplementation with *S. cumini* and *B. forficata* extracts reversed most of metabolic and developmental disorders caused by high sucrose diets. However, *S. cumini* extract was more efficient than *B forficata* in reducing hyperglycemia promoted by ingestion of both high sucrose diets and antioxidant status changes caused by 30% HSD. Overall, the obtained results highlight *D. melanogaster* as an effective model organism to investigate conditions that alter metabolic homeostasis and mainly point out the plant *S. cumini* as promising agent for studies related with metabolic disorders linked to excessive sugar.

Keywords: *Drosophila melanogaster*, *Syzygium cumini*, *Bauhinia forficata*, Sugar diet, Oxidative stress

LISTA DE ILUSTRAÇÕES

SUMÁRIO

1. INTRODUÇÃO

A Nutrição humana sofreu mudanças revolucionárias ao longo das últimas décadas em sociedades afluentes. A ingestão de alimentos tem evoluído cada vez mais em direção à alta quantidade de energia e ao desequilíbrio nutricional, traduzindo-se em um aumento dramático na prevalência de doenças crônicas que causam altas taxas de morbidade e mortalidade mundialmente (Swinburn et al., 2011; Barrera e George, 2014). Essa condição, observada principalmente nos países ocidentais, fez com que o termo "qualidade da dieta" ganhasse um enorme destaque na comunidade científica durante os últimos anos (Barrera e George, 2014). Estudos que buscam entender a relação dos nutrientes de uma dieta com funções fisiológicas, bem como a eficácia de intervenções nutricionais têm aumentado notavelmente (Patterson et al., 1994; Drewnowski et al., 1996).

Várias pesquisas já demonstraram que o tempo de vida de muitos organismos, incluindo nematódeos e roedores, é estendido quando a disponibilidade de nutrientes é experimentalmente limitada (Chapman e Partridge, 1996; Masoro, 2000; Koubova e Guarente, 2003). Em 1935 McCay e colaboradores publicaram o primeiro trabalho mostrando que a restrição alimentar prolongava o tempo de vida médio e máximo em ratos (McCay et al., 1935). Desde então, diversas pesquisas na área têm encontrado que a restrição calórica sem desnutrição, além de retardar o envelhecimento, diminui o surgimento/desenvolvimento de doenças cuja patogênese está associada com a dieta (Weindruch e Walford, 1982; Fontana et al., 2010; Masoro, 2005).

Evidências experimentais mostram que o excesso de calorias pode induzir múltiplas disfunções em uma variedade de espécies, incluindo humanos (Villegas et al., 2007; Shorupa et al., 2008). Isso porque o status nutricional, avaliado tanto pela qualidade da dieta como pela quantidade de calorias ingeridas, é um fator considerado crucial na modulação de vias metabólicas e nos processos fisiológicos que promovem a saúde e a longevidade (Birse et al., 2010). Além disso, dados da literatura mostram que alguns nutrientes específicos estão fundamentalmente implicados na patogênese de doenças crônicas e também na biologia do envelhecimento (Min e Tatar, 2006, Bruce et al., 2013). Em *Drosophila* (Mair et al., 2005; Lushchak et al, 2011) e em *Caenorhabditis elegans* (Walker et al., 2005), por exemplo, níveis elevados de carboidratos estão relacionados com processos apoptóticos e oxidativos e com declínio nas vias de reparo do DNA (envelhecimento).

As evidências de que os carboidratos podem ter efeitos adversos para a saúde tem sido um tema recorrente, com alegações de que o consumo excessivo dos mesmos aumenta significativamente o risco de desenvolver diversas patologias, entre elas obesidade, doenças cardiovasculares, diabetes mellitus (DM) e alguns tipos de câncer (Bristol et al, 1985; Burt e Pai, 2001; Johnson et al, 2007; van Baak e Astrup, 2009). Achados clínicos têm demonstrado que um padrão alimentar com restrição de carboidratos é mais eficaz que a intervenção farmacológica com metformina em reduzir as complicações do DM tipo 2 (Cater e Garg, 2002; Krook et al, 2003; Westman et al 2007; Lê e Bortolotti, 2008; Boling et al, 2009).

Já está bem estabelecido que a hiperglicemia culmina com danos oxidativos em diferentes tecidos (Forbes et al., 2008; Negre-Salvayre et al., 2009). Em termos de mecanismos, sabe-se que a auto-oxidação da glicose, a glicação de biomoléculas e a formação de intermediários (ex: metilglioxal/glioxal) e produtos finais de glicação avançada estão envolvidos no estresse oxidativo gerado via hiperglicemia (Valko et al., 2007; Ramasamy et al., 2011). Não há evidências claras sobre a capacidade de carboidratos em gerar espécies reativas de oxigênio (EROs) diretamente; mas produtos da interação de carboidratos com proteínas, lipídeos e aminoácidos podem ser geradores de EROS e promover alterações em sistemas antioxidantes (Semchyshyn et al., 2011).

Inúmeros trabalhos com DM experimental mostram que o consumo crônico de quantidades relativamente altas de carboidratos induz resistência tecidual à insulina e causa modificações oxidativas, alterando o status antioxidante de diferentes tecidos (Folmer et al., 2002; Brito et al., 2007; Lushchak et al., 2011). Além disso, pesquisas recentes, com foco em marcadores genômicos, têm estabelecido uma forte relação entre dietas ricas em carboidratos e a modulação da expressão gênica no DM (Morris *et al*, 2012; Pendse et al., 2013).

Nos últimos anos a mosca da fruta *Drosophila melanogaster* (*D. melanogaster*) tem se consolidado como um excelente modelo animal para o estudo da interação entre nutrição e os mecanismos envolvidos na patogênese de diversas doenças humanas, produzindo avanços importantes na compreensão dos efeitos da manipulação nutricional (Bier e Bodmer, 2004; Bier, 2005; Carvalho et al., 2005). Além disso, este organismo é considerado um modelo valioso para a pesquisa de genes alvos de várias patologias (de acordo com o Centro Europeu para a Validação de Métodos Alternativos (ECVAM) (Benford et al., 2000). Além de compartilhar inúmeros genes, a *D. melanogaster* e os humanos conservam vias metabólicas e sinalizadoras em comum, bem como mecanismos de regulação de ritmos circadianos e processos de aprendizagem e memória (Benton, 2008). Os mecanismos bioquímicos envolvidos no crescimento e metabolismo em *D. melanogaster* também mostram marcantes

similaridades aos de roedores e humanos (Rusten et al., 2004). Muitos dos órgãos que controlam a absorção, o armazenamento e o metabolismo em humanos também estão presentes como complexos celulares em moscas, desempenhando as mesmas funções (Scott et al., 2004).

Desde que a *Drosophila* foi introduzida como um modelo genético, há um século atrás, pesquisadores têm mantido interesse em usar dietas de laboratório para explorar conceitos básicos de nutrição (Tatar, 2011). Devido à grande importância do estado nutricional nos processos bioquímicos e fisiológicos da *Drosophila,* alterações no regime nutricional podem afetar todos os aspectos do seu ciclo de vida; isso porque o modo pelo qual esse organismo responde às variações de nutrientes na dieta se dá via mudanças nos processos de desenvolvimento e na homeostase metabólica (Markow et al., 1999, Andersen et al, 2010; Sisodia e Singh, 2012). Dietas ricas em carboidratos, por exemplo, são associadas com aumento do conteúdo de triglicerídeos em moscas adultas, indicando que a suplementação da dieta com níveis elevados de açúcar pode levar a um estado patológico na mosca (Shorupa et al., 2008).

Particularmente para o estudo de doenças metabólicas como obesidade e DM (Al-Anzi et al., 2009; Bharucha, 2009), cabe salientar que a via insulina/IGF, a qual desempenha um papel central no crescimento e no metabolismo em organismos superiores, são evolutivamente conservadas e unificadas nas moscas em uma única via (Pasco e Leopold, 2012), controlando muitos processos essenciais associados ao metabolismo, a reprodução e a longevidade (Goberdhan e Wilson, 2003; Hafen, 2004). O genoma da mosca contem um homólogo para cada componente da via de sinalização da insulina, incluindo 7 genes dilps (peptídeos insulin-like), 3 dos quais apresentam significante homologia com a insulina de mamíferos (dilps 2, 3 e 5) (Rulifson, 2002). Esses hormônios atuam através do receptor de insulina (InR) para iniciar uma cascata de eventos intracelulares mediada por componentes conservados da via de sinalização da insulina/IGF (Oldham e Hafen, 2003). Estes incluem o substrato para o receptor de insulina (Chico), o antagonista da sinalização de insulina (PTEN), proteína quinase (AKT), Fator de Transcrição da família Forkhead Box (Foxo), gene alvo da rapamicina (TOR) e outros (Figura 1) (Oldham e Hafen, 2003; Morris et al., 2012; Pasco e Leopold, 2012). Consequentemente, a expressão desses genes em *D. Melanogaster* tem sido comumente usada para avaliar a regulação do metabolismo (Bier, 2005).

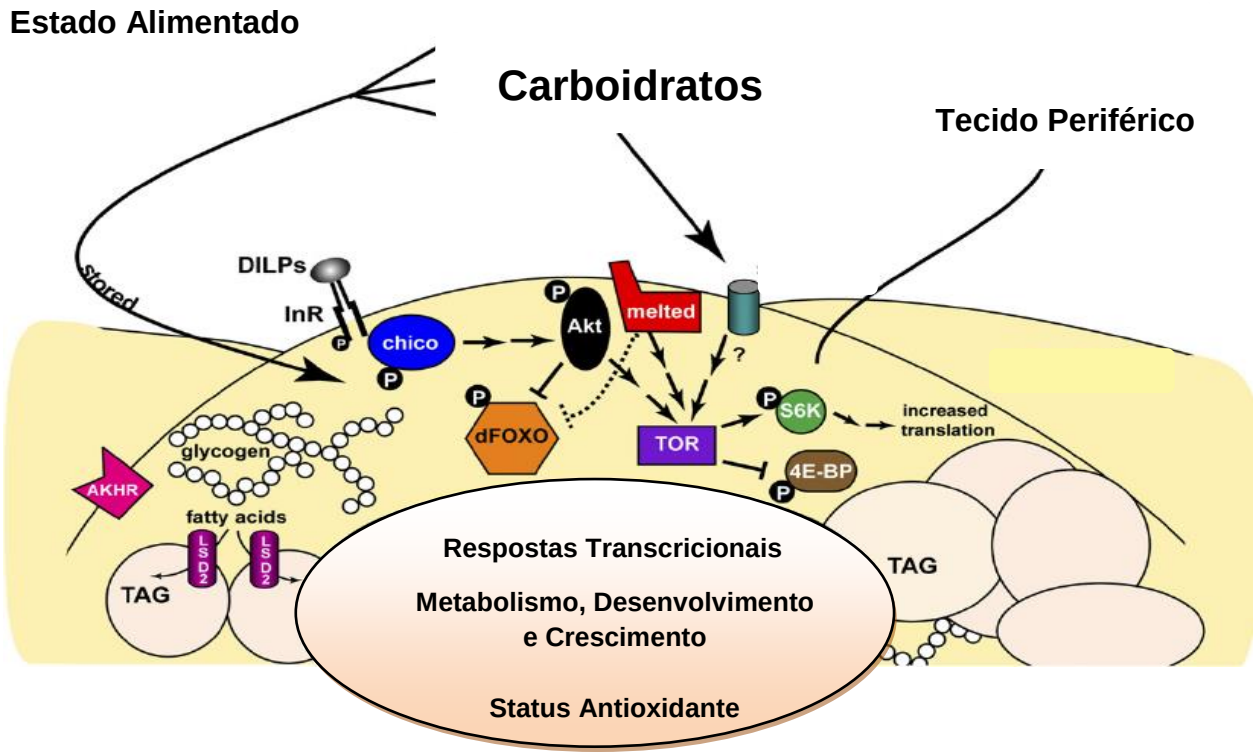

Figura 1: Representação esquemática das vias de sinalização do metabolismo em *Drosophila.* (Fonte: Adaptado de Baker e Thummel, 2007).

Devido às dificuldades encontradas em estabelecer tratamentos efetivos para o DM (Singh e Gupta 2007), o uso de produtos naturais com ação farmacológica tem sido alvo de muitas pesquisas. No Brasil, a planta *Syzygium cumini* (*S. cumini)* (L.) Skeels (Myrtaceae), sinonímia *Eugenia jambolana* (Lam.), conhecida popularmente como "Jambolão" é uma planta cujos extratos de sementes e frutos são amplamente usados na medicina tradicional como anti-hiperglicêmicos (Pepato et al., 2005; Sharma et al., 2012). Da mesma forma, a *Bauhinia forficata* (*B. forficata*) (Leguminosae), conhecida como "Pata de Vaca", é outra planta cujo extrato das folhas é usado frequentemente pela população devido às propriedades anti-hiperglicêmicas, anticoagulantes e antifibrinogenolíticas (Pepato et al., 2002; Silva et al., 2002; Oliveira et al., 2005). No entanto, não existem dados na literatura avaliando e comparando as propriedades farmacológicas dessas plantas em modelos *in vivo* de DM e/ou condições relacionadas.

Assumindo que a composição da dieta está associada com mudanças fisiológicas em um organismo, neste trabalho buscou-se avaliar se o consumo de dietas ricas em sacarose poderia induzir fenótipos consistentes com o DM do tipo 2 e estresse oxidativo em *D. melanogaster*. Concomitantemente, investigou-se o papel das plantas *S. cumini* e *B. forficata* nesses processos. Os respectivos tratamentos foram testados utizando-se larvas de *D.*

melanogaster por razões como: 1) taxa de alimentação maior quando comparada aos adultos. As larvas estão constantemente se alimentando, crescendo e armazenando energia, processos conhecidos por serem controlados pela via de sinalização da insulina (Musselman et al., 2011); 2) as larvas servem como uma sensível plataforma para avaliar anormalidades no crescimentoe 3) as larvas são sexualmente imaturas, eliminando assim as diferenças entre a fisiologia de machos e fêmeas adultos (Musselman et al., 2011).

As fases do ciclo de vida da *Drosophila*, juntamente com o desenho experimental encontram-se ilustrados na figura abaixo.

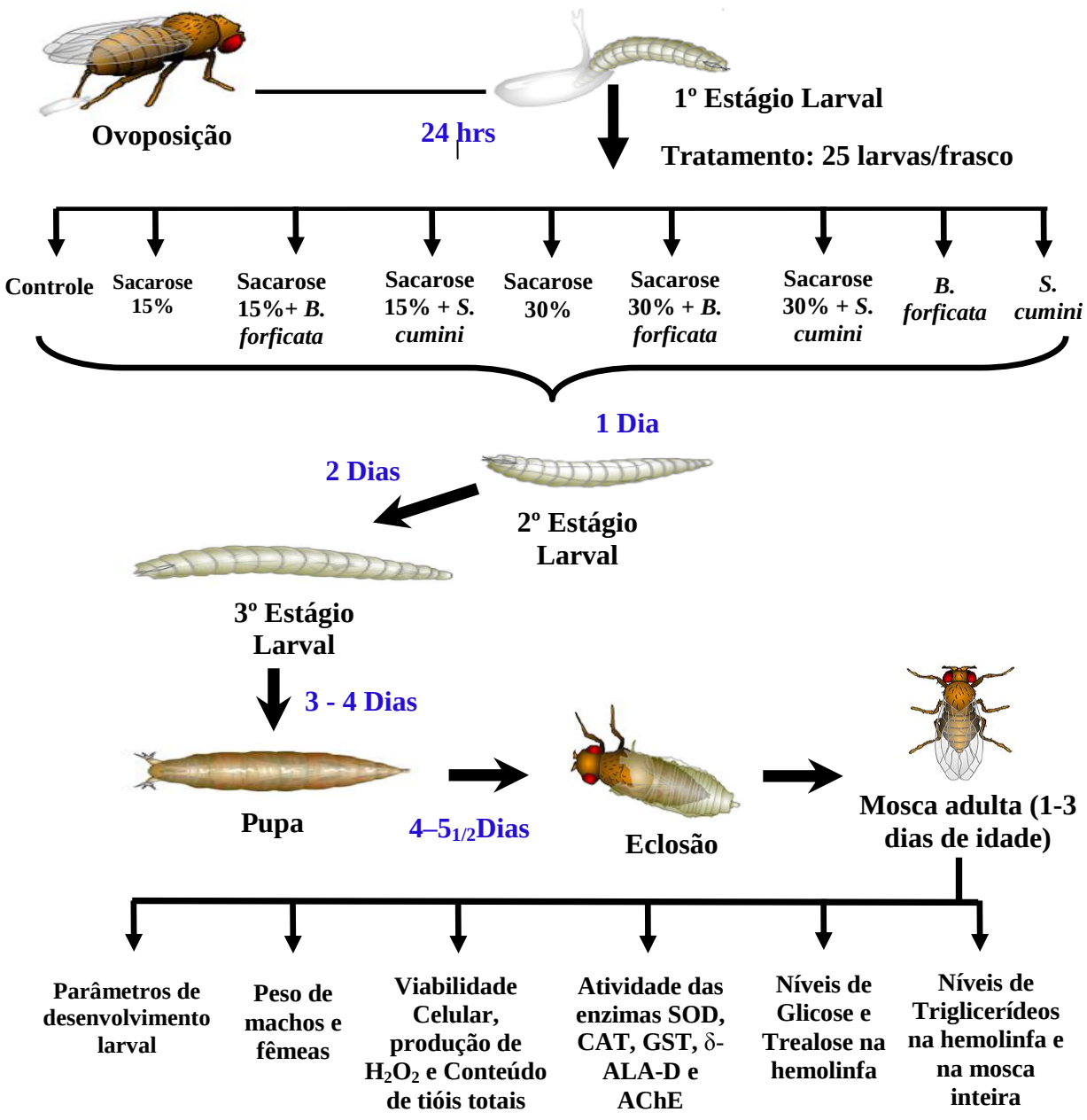

Figura 2: Desenho experimental ilustrando os diferentes estágios do ciclo de vida da mosca da Fruta, *D. melanogaster*. Fonte: Adaptado de (Elland, 2006).

2. OBJETIVOS

2.1 Objetivo Geral

Investigar se o consumo de dietas com alto teor de sacarose (15% e 30%) induz fenótipos que são consistentes com a resistência à insulina e afeta parâmetros de defesas antioxidantes em *D. melanogaster*. Os efeitos dos extratos aquosos de folhas das plantas medicinais *B. forficata* e *S. cumini* foram concomitantemente avaliados neste modelo.

2.2 Objetivos específicos

Avaliar os efeitos do consumo de dietas ricas em sacarose e dos extratos aquosos das plantas *B. forficata* e *S. cumini* sobre:

- o tempo de desenvolvimento e a sobrevivência de larvas durante o ciclo de vida da mosca *D. melanogaster* (período larval);

- o peso corporal de moscas machos e fêmeas (1 dia de idade) eclodidas das diferentes dietas;

- a viabilidade mitocondrial, o conteúdo de tióis totais e a produção de peróxido de hidrogênio (H_2O_2) em homogenato de moscas adultas eclodidas das diferentes dietas;

- a atividade das enzimas Superóxido Dismutase (SOD), Catalase (CAT), Glutationa-S-transferase (GST), aminolevulinato desidratase (δ-ALA-D) e acetilcolinesterase (AChE) em moscas adultas eclodidas das diferentes dietas;

- os níveis de Glicose e Trealose na hemolinfa de moscas adultas;

- os níveis de Triglicerídeos na hemolinfa e no homogenato de corpo total de moscas adultas.

Syzygium cumini and *Bauhinia forficata* Blunt Diabetic-Like Characteristics Induced by Feeding High-Sucrose Diets to *Drosophila melanogaster*

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Abstract

Drosophila melanogaster has been considered a suitable organism for exploring developmental/metabolic dysfunctions and therapeutic strategies. Here we used *Drosophila melanogaster* to evaluate the role of plants *Syzygium cumini* (*S. cumini*) and *Bauhinia forficata* (*B. forficata)* on the effects triggered by consumption of high-sucrose diets (HSD), namely: stress oxidative markers and phenotypic responses associated to insulin signaling. The larvae were fed with HSD, containing 15 or 30 % of sucrose (15% HSD and 30% HSD) supplied or not with aqueous extracts of *S. cumini* and *B. forficata* (5mg/mL). Thereafter, 1 day-old newly hatched flies from treated larvae were used to assess biochemical parameters. During the larval stage, 15% and 30% HSD intake delayed the time to pupation and reduced the number of white pupae. These effects were accompanied by increased mortality of emerged flies from both diets. 15% and 30% HSD intake substantially increased the levels of glucose+trehalose and triglycerides in hemolymph of adult flies. The flies from larvae fed on 30% HSD also had a significant reduction in the body-weight compared to the control. The intake of both sucrose diets elevated the levels of H_20_2 in adult flies. Indeed, larval ingestion of 30% HSD induced mitochondrial viability loss and disruptions in the catalase, superoxide dismutase, gluthatatione- S-transferase, acetylcholinesterase and δ-aminolevulinate dehydratase activities in adult flies. Importantly, *S. cumini* and *B. forficata* blunted most of the developmental/metabolic dysfunctions elicited by HSD. In general, *S. cumini* was more efficient than *B. forficata* in reducing the hyperglycemia and in blunting the imbalance on the antioxidant status elicited by HSD. Our findings point *Drosophila melanogaster* as a reliable tool to investigate conditions that disrupt the fuel metabolic homeostasis; and highlight the plant *S. cumini* as a promising candidate for further studies on the search for therapeutic strategies to treat metabolic disorders linked to dietary high sugar intake.

Keywords: *Drosophila melanogaster, Syzygium cumini, Bauhinia forficata*, *Sugar diet, Sucrose, Oxidative stress*

1. Introduction

It is well known that calorie restriction extends lifespan and prevents age-related disorders while dietary caloric excess induces multiple metabolic dysfunctions in a wide array of species, including humans [1-4]. Epidemiological studies have shown that the diet components are the most important environmental risk factors for the development of chronic metabolic diseases [5, 6]. Furthermore, epidemiological and experimental studies have pointed out the importance of the diet composition as key factor in the prevention and/or treatment of chronic diseases [2, 3, 6]**.** Regarding to carbohydrates, there is evidence that increased consumption of sucrose/fructose-rich diets contributes to the overnutrition-triggered metabolic changes as obesity and insulin resistance, which are important hallmarks of type 2 Diabetes mellitus (DM) [7, 8]. According to the World Health Organization, the prevalence of type 2 DM has increased dramatically worldwide and will affect about 400 million adults in 2030 [9].

The underlying mechanisms for the long term complications of type 2 DM are still poorly understood. However, accumulating evidence strongly points to the hyperglycemia as a potent inducer of cell damage via oxidative stress [10, 11]. In fact, biochemical pathways activated under hyperglycemic condition such as glucose autooxidation, protein glycation and advanced glycation end products (AGEs) formation can promote oxidative damage by promoting free radical generation and decreasing the antioxidant status of cells [10-17]. Knowing that oxidative stress plays a central role in the cytotoxicity elicited by hyperglycemia, various studies have been conducted to investigate the antioxidant and/or hypoglycemic potential of different agents in experimental DM models.

In recent years the fruit fly *Drosophila melanogaster* (*D. melanogaster*) has emerged as an advantageous alternative organism to expensive mammalian models and a promising model system for exploring different human pathologies, including metabolic disorders as obesity and DM [18-21]. Many biochemical mechanisms involved in the control of growth and metabolic processes in humans are present in the fly [22, 23]. Of particular importance, various neuroendocrine architecture and mechanisms in *D. melanogaster* resemble those found in mammals [24]. Similarly, *D. melanogaster* has insulin-producing cells (IPC), insulin-like peptides (ILP) and receptor (InR); conserving the molecular insulin/insulin-like growth factor signaling pathways [25, 26, 27]. Specially regarding to DM, literature data have demonstrated

that high sugar diet intake elicits insulin-resistant phenotypes in *D. melanogaster* that represent the pathophysiology of type 2 DM in humans [21, 28]. These phenotypes are normally characterized by elevated fat deposition and circulating glucose, systemic insulin resistance, shortened fecundity and life-span [21, 27, 28, 29].

Based on the fly system as an important genetic tool, studies have been carried out to investigate the possible interaction between caloric diets and gene expression on type 2 DM [21, 29]. However, experiments using *D. melanogaster* to evaluate biochemical parameters related to oxidative stress induced by high-sugar diet intake are scarce in the literature [15]. Consequently, *D. melanogaster* is an attractive organism model for studying diet-induced metabolic disorders and potential therapeutic strategies to remedy them.

In conventional therapy, type 2 DM is usually treated with different synthetic types of hypoglycemic drugs. However, many people around the world have been using folk medicinal plants and herbs to treat metabolic diseases, including DM [30]. In several cases, the use is empirical and without any experimental scientific support. In Brazil, the plants *Bauhinia forficata* Link (*B. forficata,* Leguminosae) known as Cow Hoof and *Syzygium cumini* (L) Skeels (*S. cumini*, Myrtaceae *syn: Eugenia jambolana*) known as Jambolan or Java plum, are popularly used as hypoglycemic agents in folk medicine [31]. Apart from South America, these two plants are found in Eastern Africa and Asia [32, 33]. In the form of infusions and decoctions, the leaves are the part of plants more commonly used by population [32]. However, few studies have investigated the beneficial effects of *S. cumini and B. forficata* leaves and/or compared them in *in vivo* models of DM or related conditions [34]. Considering the importance of more detailed information about the potential anti-diabetogenic action of *S. cumini* and *B. forficata* leaves, the present study was delineated to evaluate the effects of these plants on the antioxidant status and phenotypic responses consistent with type 2 DM in *D. melanogaster* fed with high-sucrose diets.

2. Material and Methods

Chemicals: 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Ampliflu red fluorescent dye and Horseradish peroxidase, quercetin, rutin and kaempferol were obtained from Sigma

Chemical Company (St Louis, MO, USA). Acetic acid, gallic acid, chlorogenic acid and caffeic acid purchased from Merck (Darmstadt, Germany).

Leaves extracts preparation*: Bauhinia forficata* and *Syzygium cumini* leaves were collected in the Jardim Botânico of the Universidade Federal de Santa Maria (UFSM). The material was identified and authenticated in the herbarium of the Institution. The plant leaves were dried (5% humidity, room temperature), powdered and then prepared in infusion at concentration of 30% (30g of powder/ 100 ml of water) for 30 minutes, similarly to the folk-medicine preparation method. After that, the samples were filtered and subjected to lyophilization. The resultant powder was dissolved in water to prepare the solution to be added in the diet of flies.

Quantification of compounds by HPLC-DAD: High performance liquid chromatography (HPLC-DAD) was performed with the HPLC system (Shimadzu, Kyoto, Japan), Prominence Auto Sampler (SIL-20A), equipped with Shimadzu LC-20AT reciprocating pumps connected to the degasser DGU 20A5 with integrator CBM 20A, UV-VIS detector DAD (diode) SPD-M20A and Software LC solution 1.22 SP1.

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 2% acetic acid and methanol, following the method described by Laghari et al. (2011) [35] with some modifications. The lyophilized aqueous extract of leaves from *S. cumini* and *B. forficata* were analyzed, dissolved in water at a concentration of 5 mg/mL. 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 0.050 – 0.250 mg/ml for quercetin, rutin and kaempferol, and 0.006 - 0.250 mg/ml for gallic, chlorogenic and caffeic acids. The quantification was carried out by integration of the peaks using the external standard method, at 254 nm for gallic acid, 325 nm for caffeic and chlorogenic acids, and 365 nm for quercetin, rutin and kaempferol. The flow rate was 0.8ml/min and the injection volume was 50µl. The chromatography peaks were confirmed by comparing their retention time and Diode-Array-UV spectra

with those of the reference standards. All chromatography operations were carried out at ambient temperature and in triplicate.

Fly strain and culture conditions*: D. melanogaster* wild-type was obtained from National Species Stock Center, Bowling Green, OH, USA. The flies were kept in a humidified (60%), temperature-controlled incubator with 12 hour on/off light cycle at 24 °C in 2,5 x 6,5 cm bottles containing 10mL standard cornmeal medium. All experiments were performed from synchronized first instar larvae (L1) originated from the same strain. Larval period was chosen for the ingestion treatments due the larvae present higher feed rate, rapid growing and sexual immaturity as compared to adults. Indeed, larval period also serve as a platform for evaluating relevant growth failures.

Larval treatment: For larvae collection, approximately 100 female flies were allowed to lay eggs in different vials containing a medium enriched with yeast to ovoposition during a period of 6 hours. After 24 hours, 25 first instar larvae (L1) were rinsed in 0,5% (v/w) sodium hipochloride solution and PBS 1% and then transferred to the culture medium from each treatment with a histological needle. This asepsis method was realized to reduce the amount of microorganisms that could proliferate in adherent culture medium and interfere in the larval growth and survival [36]. Larvae were monitored daily, following the changes of development on the larval-pupa and pupa-adult fly stages.

For the experiments, the larvae were fed with a standard food (medium: 1%, w/v brewer's yeast; 1%, w/v powdered milk; 1%, w/v agar, 0,08% v/w nipagin (methyl-p-hydroxybenzoate)) containing different sucrose concentrations (2, 15 and 30% w/v) and aqueous leaves extracts of *B. forficata* and *S. cumini* at final concentration of 5 mg/mL medium. The choice of this concentration was based in our previous toxicological tests, which showed that both extracts in the range of 0,1-10 mg/mL medium did not cause signals of toxicity in flies (data not shown). Thus, the larvae were divided into 9 groups: Control (2% sucrose), 15% HSD (15% high-sucrose diet); 30% HSD (30% high-sucrose diet); *B. forficata; S. cumini*; 15% HSD plus *B. forficata*; 30% HSD plus *B. forficata*; 15% HSD plus *S. cumini* and 30% HSD plus *S. cumini*. The treatments were maintained during all larval period. The newly-hatched flies (1-3 days) were used for biochemical analysis.

B. forficata and *S. cumini* groups were used in all experiments. In order to clarify, these results were not showed in the figures because *S. cumini* and *B. forficata*, given alone, did not alter the parameters evaluated when compared to the control group (Data not shown).

Development and growth parameters assessment

Developmental time course: The larval development was measured according the method of Pasco and Leopold (2012) [28], by assessing the time from first instar larvae (L1) to white pupal stage (metamorphosis). Then, L1 larvae, collected 24 hr after egg deposition, were observed daily and white pupae number was counted every day at 14:00 hr. The number of white pupae was expressed as percentage in relation to the control.

Larvae survival rate: The larvae survival rate was evaluated by counting daily of the number of flies emerged from viable larvae that survived during the developmental stages.

Metabolic Parameters

Flies weight: The flies (1-day-old) hatched from larvae were separated in groups containing 5 female and 5 males and then weighed on a ME5 Sartorius® microbalance.

Circulating carbohydrates and triglycerides measurements: Adult flies hemolymph was collected according the protocols given by Sigma Aldrich, with few modifications. Flies with wings removed, were placed in 0.5 mL eppendorfs containing small holes and centrifuged within the 1.5 ml eppendorfs (5 min, 5000 rpm, at 4°C). To obtain 0,5 µL of hemolymph for assays, approximately 30 flies were used. Hemolymph was 50-fold diluted in glucose/trehalose buffer (5 mM Tris [pH 6,6] 2,7 mM KCl, 137 mM NaCl) [37] or triglycerides buffer (0,02 M TFK [pH 7,4] + 0,5% Tween 20) and heated for 5 min at 70 ºC. First, glucose and triglycerides were measured after incubation at 37ºC for 25 min using colorimetric assay kits and processed as per the manufacturer's instructions. Trehalose, a glucose disaccharide synthesized from intracellular glucose in fat body cells and secreted into the hemolymph, was measured after digestion with porcine trehalase (Sigma, T8778).

1µL of trehalase was added in 30 µL of the diluted hemolymph and incubated at 37ºC overnight, for converting trehalose into glucose, and the total amount of resulting glucose was measured. Trehalose levels were obtained by subtracting the total free glucose present in the sample treated with trehalase from samples without the enzyme. Carbohydrate and triglycerides levels were estimated against a calibration curve of standard solutions.

Whole body triglycerides measurement:

Whole flies were weighed and then homogenized in 0,5% Tween 20 + TFK (0,02M pH 7,4), 1:10. The homogenate was centrifuged at 13000 rpm for 3 min to remove particulates that could interfere with the colorimetric assays. The supernatant was immediately heated for 5 minutes at 70ºC to inactivate enzymes [38]. Ten µL of this homogenate was mixed with a specific medium for triglycerides measurement according to supplier´s instructions (Sigma Kit assay).

Biochemical assays

Whole-fly tissue homogenate preparation: Briefly, ice-anesthetized 1-3 day old flies (both gender) were weighed in groups of 20 and manually homogenized in phosphate buffer 0,02 M, pH 7,4. Following centrifugation (appropriate speed and time to each test) at 4ºC, the supernatant (S_1) was maintained on ice until the respective biochemical assays. For determination of cell viability, total thiol content and hydrogen peroxide production in the supernatant, the whole-fly homogenate was centrifuged at 3500 rpm for 5 min. Except to ALA-D activity, for all other stress oxidative markers the rotation used was 7500 rpm.

Mitochondrial viability evaluation: Mitochondrial **v**iability was evaluated by dehydrogenase activity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay, according to the method described by Babot *et al.* (2005) [39]. The formazan, product formed by incubation of the supernatant samples with MTT, was solubilized in dimethyl sulfoxide (DMSO) and measured at 570 and 630 nm. Values were standardized per protein content and expressed as percentage in relation to the control.

Total thiol determination: Total thiol content was determined based on a spectrophotometric method proposed by Ellman (1959) [40] using DTNB reagent (5,5-dithiobis (2-nitrobenzoic acid), which reacts with SH groups. Colorimetric analysis was carried out at 412 nm and the content of SH groups was calculated regarding to the values found for known concentrations of GSH used as standard. Thiol levels were standardized per protein content and expressed as percentage in relation to the control.

H_2O_2 production measurement: Supernatant H_20_2 content was detected using the red fluorescent dye Amplifu in the presence of horseradish peroxidase enzyme according to Votyakova and Reynolds (2001) [41]. Horseradish peroxidase (0.2 U/ ml) and Amplifu red reagent (1 mM) were added to the medium and then, an aliquot of supernatant was applied. The fluorescence equivalent to H_20_2 content was detected at 30°C in a Shimadzu spectrophotometer with excitation and emission fluorescent wavelengths of 550 and 585nm (slit 3 and 5) respectively. The positive control signal was produced by the addition of known amounts of H_2O_2 at the end of each experiment.

Superoxide dismutase (SOD) activity determination: SOD activity was determined spectrophotometrically according to the method proposed by Kostyuk and Potapovich (1989) [42], based in the ability of SOD in inhibiting quercetin auto-oxidation at pH 10.0 in the presence of TMED (N, N, N1 , tetrametiletilenediamina N1) and 0.02 M phosphate buffer with 0.08 mM EDTA. Kinetic analysis of SOD activity started from quercetin addition and the reaction was monitored at 406 nm for 20 minutes at 25ºC. The results were corrected by the amount of protein in the supernatant and calculated as percentage of inhibition of quercetin oxidation.

Catalase (CAT) activity: CAT activity was determined spectrophotometrically by method of Aebi (1984) [43], based in the ability of CAT to degrade H_2O_2, with some modifications. Kinetic analysis of CAT activity started from the addition of H_2O_2 to the reaction medium along with S1. The decrease in the optical density at 240 nm (OD240) was measured over 2 min at 25°C and the results were linear with regard to time and amount of S1. The activity was expressed as units of CAT per mg protein (U/mg protein).

Glutathione-S-Transferase (GST) activity: GST activity was determined according to the method described by Habig et al. (1974) [44], adapted to microplates. This method is based on the principle that GST enzyme catalyzes the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) to reduced glutathione (GSH), originating a thioether (S-2, 4-dinitrophenyl glutathione) which can be monitored by the increase in absorbance at 340nm. The molar extinction coefficient used for CDNB was 9.6 mM^{-1} cm^{-1}. The results were expressed as milliunits of enzyme activity/mg of protein (mU/mg protein).

Delta-aminolevulinate dehydratase (δ-ALA-D) activity: The δ-ALA-D activity was determined according to the method proposed by Sassa (1982) [45], by measuring the rate of porphobilinogen (PBG) formation with some modifications. About 80 adult flies were homogenized in 210 µl of 5 mM Tris HCl, pH 8.5, and centrifuged at 4000 rpm for 10 minutes. An aliquot of the supernatant S1 was incubated at 37°C for 3 hours with 5-aminolevulinic acid (ALA). The reaction product was determined at 555 nm using Ehrlich reagent, which upon reacting with the PBG produces a pink color. Enzyme activity was calculated as nmol of PBG formed per mg protein per hour (nmol PBG/mg protein/hour).

Acetylcholinesterase (AChe) activity: AChe activity was measured as described by Ellman et al. (1961) [46], adapted to microplates. Briefly, aliquots of supernatant (20 µL) were incubated at 30°C for 2 min with 0.1 M phosphate buffer, pH 7.4, 10 mM DTNB as chromogen. After 2 min, the reaction was initiated by the addition of acethylthiocholine (8 mM) as substrate for the reaction mixture. Absorbance was determined at 412 nm during 2 min. Enzyme activity was calculated as µmol of acethylthiocholine hydrolyzed mg $protein^{-1}$ min^{-1}.

Protein determination: The protein content in the whole body homogenates was determined by the method of Lowry *et al* (1951) [47].

Statistical analysis: The larval development data were analyzed by non-parametric methods using Mann-Whitney test (GraphPad InStat3 Program). All other results were analyzed by Two-way ANOVA followed by Duncan Multiple Range Test when appropriate. Differences between groups were considered significant when $p < 0.05$. The graphics were made using the GraphPad Prism (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 HPLC analysis

HPLC fingerprinting of *B. forficata* lyophilized aqueous extract revealed the presence of gallic acid (6.53%, peak 1), chlorogenic acid (2.08%, peak 2), caffeic acid (1.72%; peak 3), rutin (0.91%; peak 4), isoquercitrin (4.45 %; peak 5), quercetin (7.19%; peak 6) and kaempferol (2.30%; peak 7) (Figure 1A). In the chromatograms of *S. cumini*, it was possible identify the presence of gallic acid (3.46%; peak 1), chlorogenic acid (2.09%; peak 2), caffeic acid (1.57%; peak 3), rutin (4.95%; peak 4), quercetin (3.37%; peak 5) and kaempferol (0.62%; peak 6) (Figure 1B).

3.2 Instar larvae developmental time and L1 Larvae Survival rate

The intake of both high-sucrose diets retarded significantly the larval development by delaying the time to the first pupation (2-3 days) and decreasing, throughout the days (± from day 5), the number of white pupae in relation to the control group (Fig 2A and 2B). Simultaneous exposure to *B. forficata* blunted completely the effects induced by 15% HSD intake on the white pupae during all experimental period (Fig 2A). The beneficial effect of *S. cumini* in this parameter (± from 13 until 18 day) was less pronounced than *B. forficata*.

Both treatments attenuated the effect of 30% HSD intake on the number of white pupae, but not to the control level (Fig 2B). Again, *B. forficata* was more efficient (from 8 to 18 day) than *S. cumini* (from 15-18 day) when compared to the 30% HSD group.

The intake of both diets rich in sucrose diminished the survival rate of flies (Fig 2C and D). The percentage of flies emerged from 15% HSD and 30% HSD, when compared to the control group, was respectively 50% and 25% ($p<0.05$). *B. forficata* and *S. cumini* protected the flies against the mortality provoked by high-sucrose

diets. However, only the effect 15% HSD-induced was completely restored to the control levels. The panels E and F show representative images of L3 larvae and pupa from the different groups (Figure 2E and 2F).

3.3. Body Weight of the flies hatched from larvae grown on high-sucrose diets and treated with *S. cumini* and *B. forficata* extracts

The 15% HSD intake did not change the body weight of female and/or male flies when compared to the respective control groups (Figure 3A). Differently, the flies from larvae fed on 30% HSD had a significant decrease in the body weight, when compared to the control (Figure 3A; $p<0.05$). The loss of body mass of female and male was equivalent to 22 and 20% respectively (Figure 3B). These effects were significantly blunted by both *S. cumini* and *B. forficata* treatments. The figure 3 (C and D) also shows representative images of the whole body and wing size of flies from different groups.

3.4 Effect of *S.cumini* and *B.forficata* extracts on glucose and triglycerides levels of flies emerged from larvae fed on high-sucrose diets.

The consumption of diets rich in sucrose increased substantially hemolymph levels of glucose+trehalose in adult flies ($p<0.05$). The flies hatched from larvae fed on 15% HSD had glucose+trehalose levels 28.5% higher than control flies; and the flies fed with 30% HSD had an increase of 53.7% in hemolymph sugar levels when compared to the control group (Figure 4A-B). This hyperglycemic effect associated with high-sucrose diets intake was blunted only by *S. cumini*.

The levels of triglycerides verified in whole body homogenate of flies from 15% HSD and 30% HSD were respectively 1,4 and 2-fold higher than the values found in the control group (Figure 4C and D). Similarly, an increase of 1,7 and 2,5-fold was found in hemolymph levels of triglycerides in flies fed with 15% HSD and 30% HSD respectively, when compared to the control (Figure 4C and 4D). *S. cumini* restored the levels of triglycerides in hemolymph increased by both sugar diets and in whole body samples induced by 30% HSD. *B. forficata* reduced only the hemolymph levels of triglycerides elevated by 30% HSD. Overall, *S. cumini* was more effective than *B. forficata* in mitigating the biochemical impairments induced by high-sucrose diets.

3.5 Mitochondrial viability and H_20_2 production determination

Intake of the 30% HSD by flies caused a significant loss of mitochondrial viability, when compared to the control (Figure 5). This was indicated by the decrease in mitochondrial MTT reductive ability. Only *S. cumini* was able to blunt this effect elicited by diet at control level (p<0.05). 15% HSD intake did not affect *per se* this parameter.

The figure 6 shows that the H_20_2 levels were markedly increased in homogenate of flies hatched from larvae grown on 15% HSD and 30% HSD when compared to the control (an increase of approximately 34% for both diets). Again, only *S. cumini* was efficacious in blunting these effect induced by high-sucrose diets (p<0.05).

Antioxidant parameters

3.6 SOD, CAT and GST enzymes and Thiol content

The activity of antioxidant enzymes SOD, CAT and GST was disrupted only in flies emerged from larvae fed on 30% HSD. This diet intake caused an increase of approximately 23% in CAT activity; and a reduction of approximately 40.4 and 28.9% in SOD and GST activities respectively, when compared to the values found in the control group (Figure 7A-C). All changes elicited by 30% HSD on the antioxidant enzymes were blunted by *S. cumini.* Except for SOD, *B. forficata* reduced the effects of 30% HSD on the activity of antioxidant enzymes.

There is no difference among the groups on the total thiols content (data not shown).

3.7 AChe and δ-ALA-D Activities

Flies derived from larvae fed on 30% HSD had a marked decrease in the activity of AChe and δ-ALA-D when compared to the control group (Figure 8A and 8B). *S. cumini*, but not *B. forficata*, restored to the control levels the activity of both enzymes (Figure 8 A-B). 15% HSD did not inhibit AChe or δ-ALA-D activities.

4. Discussion

The growing impact of high morbidity and mortality rates worldwide associated to metabolism-related diseases has potentiated the interest by role of nutritional components in the etiology of the metabolic syndromes as well as the understanding of the mechanisms that underlie metabolic regulation. In parallel, the use of invertebrate organisms for exploring the physiological consequences of metabolic disorders has been extensively applied in the last decades. In this scenario, *D. melanogaster* has emerged as an important alternative model to vertebrates due its well documented metabolic, growth and genetic molecular machinery [19, 26].

Herein, *D. melanogaster* larvae were used as model organism for exploring the detrimental effects triggered by consumption of diets rich in sugar. In *D. melonogaster* life cycle, the larva stops feeding and initiates pupation at a very specific time after hatching, and this fact can be used as an important developmental transition point to evaluate alterations in growth patterns [48]. So, using *D. melonogaster* larvae, we analyzed the potential beneficial role of two plants, which are popularly used to treat DM related problems, on the effects elicited by high-sucrose diets intake. Here we investigated oxidative stress markers and some phenotypic responses that are considered dependent on insulin signaling as developmental time, survival, growth and body weight [49, 50]. In general, high-sucrose consumption negatively affected the larval development and diminished the survival rate of flies emerged from larvae. These effects were accompanied by elevated hemolymph glucose+treaholose and triglycerides levels, weight body loss and signs of cellular events possibly mediated by oxidative stress. Interestingly, most of these harmful responses were blunted by both plants. However, *S. cumini* was more effective than *B. forficata* because its protective effects encompassed all parameters analyzed (developmental/metabolic and antioxidant status).

Some studies with *D. melonogaster* have been published a direct relation between high-sugar feeding and the development of phenotypes insulin signaling pathways-induced [21, 27, 28, 29]. Although most of them were conducted with adult flies, recent evidence has indicated that larvae fed with a high-sugar diet can develop phenotypic characteristics linked to insulin signaling pathways, for instance, shortened lifespan, reduced size and delayed development, when compared with those reared on control food [27, 28]. In accordance, our results show that the

consumption of 15% and 30% HSD delayed the time from first instar larvae to first pupation and decreased the white pupae percentage. Simultaneously, the 30% HSD also caused a marked decrease on the body weight of the flies.

In order to investigate whether high-sucrose diets could lead to the disruption of metabolic homeostasis, we evaluated sucrose influences on both carbohydrate and fat levels in adult flies derived from larvae fed on the high-sucrose diets. Insects contain two main types of sugar in circulation: glucose and trehalose. Glucose is obtained from diet, and trehalose is used as a homeostatic molecule that originates from fat body and is used to distribute sugar to peripheral tissues [51]. We observed that flies from both high-sucrose diets exhibited elevated hemolymph glucose+trehalose levels. Concomitantly, the intake of both diets increased the whole-body and hemolymph triglycerides levels. Taken together, these results indicate that excess dietary sucrose produced important features of type 2 DM, for instance, hyperglycemia and hypertriglyceridemia. These detrimental effects of high-sugar diets on metabolic homeostasis parameters were similar to those observed in the recent studies performed by Musselman (2011) [27] and Pasco and Léopold (2012) [28]. In addition, those authors verified an increase of DILPs (insulin-like peptides) expression on larvae upon feeding a high-sucrose diet. Insulin-like peptides in *D. melonogaster* (particularly Dilps 2, 3 and 5) share sequence, structural and functional similarities with vertebrate insulin-like growth factor and insulin, regulating both growth and glucose homeostasis [27]. This fact points the ability of the respective diet to induce T2D-like phenotypes and promotes insulin resistance in the flies. Therefore, the data obtained here support findings showing that high-sugar diets induce type 2 DM features via modulation of insulin machinery in flies [21, 27, 28, 29].

Considering that the high-sucrose diets were associated with a hyperglycemic state and that this condition triggers oxidative tissue damage [13, 27, 28], we focus our investigation to evaluate some oxidative stress markers in flies. The intake of both high-sucrose diets was associated with hydrogen peroxide overproduction. Indeed, 30% HSD, but not 15% HSD, elicited simultaneously a reduction in the mitochondrial viability and disruptions in the activity of antioxidant enzymes SOD, CAT and GST. The intake of 30% HSD inhibited SOD and GST activities and increased CAT activity. As these enzymes are considered the first line of defense against oxidative stress in *D. melonogaster* [15, 52], disruptions in their activities

could underlie the effects triggered by 30% HSD in mitochondrial viability and H_20_2 production. Although there are few data in the literature investigating oxidative parameters in flies derived from larvae grown on high-sucrose diets; our findings are in agreement with a recent study showing that adult flies from larvae fed on diets rich in glucose and fructose developed oxidative stress, namely: elevated levels of lipid peroxidation and protein carbonyls and disruptions on the SOD and Catalase enzymes activities [15]. Besides, the developmental and survival perturbations observed in the high-sucrose feeding larvae can be connected with oxidative stress induction.

In *D. melonogaster*, oxidative stress may compromise ecdysone hormone signaling. This hormone regulates the progression through larval stages and pupal metamorphosis; and its disruption in response to redox imbalance can alter the time of these lifecycle events [53]. Although this was not investigated here, we can suppose that ecdysone and related processes might be involved in the negative effects of high-sucrose diets on the developmental and survival parameters.

From our knowledge, this is the first study that investigated the relation of sucrose diets intake and the activity of delta-aminolevulinate dehydratase (δ-ALA-D) enzyme in flies. This sulfhydryl enzyme participates in the porphobilinogen formation and its measurement used together with other biomarkers constitutes an important parameter of oxidative stress [13, 54]. Studies with other species have demonstrated that δ- ALA-D activity is significantly inhibited under hyperglycemic condition via glycation of its active site lysine residues [55, 56]. In fact, literature data show a decreased activity of δ-ALA-D in diabetic patients and in mice feeding with high sucrose and/or glucose diets [13, 55, 56]. In analogy, in our study, the flies that received the diet containing 30% sucrose, at larval period, had an inhibition of δ-ALA-D activity in adulthood. Considering all responses developed by flies exposed to high-sugar diets, it is reasonable assume that the inhibition on δ-ALA-D activity may be consequence of oxidative/glycation events induced by hyperglycemia.

It has been reported that defects in insulin pathway affect brain function and alter neurotransmitter systems in *Drosophila* [57, 58]. Here, the abnormal nervous system function was assessed by measuring the cholinesterase activity since acetylcholine is considered the main excitatory neurotransmitter in fly brains [57]. We found that the intake of 30% HSD reduced significantly AChe activity. These findings are in conformity with literature data showing that cholinesterase activity is

diminished in insulin mutant flies [58]; and support the idea that the ingestion of high-sucrose diets may disrupt insulin signaling.

In Brazil folk medicine, the leaves of *S. cumini* and *B. forficata* plants are commonly used in different phytopreparations to lower blood glucose levels [59, 60, 61]. In addition to hypoglycemic property, some studies have indicated that the leaves aqueous extract of *S. cumini* and *B. forficata* exhibit antioxidant activity [62, 63, 64]. Nonetheless, there are few data about the action mechanisms and the efficacy of the both genus in *in vivo* experimental models. So, our results show, for the first time, the effectiveness of *S. cumini* and *B. forficata* in reducing the deleterious effects induced by high-sucrose diets consumption using *D. melanogaster* as organism target. *S. cumini* and *B.forficata* were similarly effective in blunting the changes on developmental parameters mediated by high-sucrose diets; however, *S. cumini* extract was more efficient in normalizing the elevated levels of glucose and triglycerides and the imbalance on the antioxidant/oxidant status elicited by diets.

In this way, there is evidence that *S. cumini* reduce oxidative damage in diabetic rats by restoring glutathione levels, SOD, CAT, GPx, GST activities and decreasing lipid peroxidation [65, 66]. Likewise, *in vitro* and *in vivo* evidence has pointed the antioxidant potential of components from *B. forficata* leaves [62, 63]. Usually the natural antioxidants present in the chemical composition of leaves from both plants are highlighted as responsible for their beneficial effects. In our samples, HPLC analysis revealed that flavonoids and phenolics are the major components of the leaves extracts.

In summary, this study shows that high-sucrose diets elicited in *D. melanogaster* phenotypic responses consistent with insulin signalizing disturbances and redox status imbalance. Besides, our findings confirm and point the utility of *D. melanogaster* as a powerful tool to investigate therapeutic strategies on metabolic disorders as well as highlight mainly the plant *S. cumini* as a promising candidate to treat diabetic symptoms. However, the demonstration of signaling pathways triggered by hyperglycemia/insulin resistance in this model deserves further investigations.

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6. Legends

Figure 1. (A) Representative high performance liquid chromatography profile of *B. forficata* extract: Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rutin (peak 4), isoquercitrin (peak 5), quercetin (peak 6) and kaempferol (peak 7); **(B)** Representative high performance liquid chromatography profile of *S. cumini* extract: Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rutin (peak 4), quercetin (peak 5) and kaempferol (peak 6). Chromatographic conditions are described in the Methods section.

Figure 2. Effect of *S. cumini* and *B. forficata* treatments on developmental parameters of larvae fed on high-sucrose diets (HSD). **(A,B)** Time from first instar larvae to the first pupation, represented by cumulative number (%) of white pupae among the experimental days; **(C,D)** % survival of L1 larvae, evaluated by total number of flies hatched from larvae during all experimental period. % white pupae data were evaluated by Mann-Whitney test. % survival data are presented as mean±S.E.M by Two-way ANOVA followed by Duncan Test. *p<0.05 indicates statistical difference from control; $^{\#}p$<0.05 indicates statistical difference from respective HSD. Representative images of wandering L3 larvae **(E)** and pupa collected during the developmental stages **(F)**.

Figure 3. Effect of *S. cumini* and *B. forficata* on weight-body of flies hatched from larvae fed on high-sucrose diets (HSD). Weight of 1 day-old females and males emerged from larvae fed on 15% HSD **(A)** and 30% HSD **(B)** and treated with *S. cumini* and *B. forficata* aqueous extracts. Data are presented as mean±S.E.M. Two-way ANOVA followed by Duncan Test. *p<0.05 indicates statistical difference from control; $^{\#}p$<0.05 indicates statistical difference from 30% HSD. Representative images: **(C)** Whole body and **(D)** wing sizes of 1 day old females.

Figure 4. Effect of *S. cumini* and *B. forficata* on carbohydrate and fat content of flies hatched from larvae fed on high-sucrose diets. **(A,B)** Glucose+trehalose levels from hemolymph **(C,D)** Triglycerides levels from hemolymph and whole-body. Data are presented as mean±S.E.M by Two-way ANOVA followed by Duncan Test. *p<0.05

indicates statistical difference from control; $^{\#}p<0.05$ indicates statistical difference from respective HSD.

Figure 5. Effect of *S. cumini* and *B. forficata* on mitochondrial viability in homogenates of adult flies hatched from larvae fed on high-sucrose diets. Data are presented as mean±S.E.M by Two-way ANOVA followed by Duncan Test. *$p<0.05$ indicates statistical difference from control; $^{\#}p<0.05$ indicates statistical difference from HSD.

Figure 6: Effect of *S. cumini* and *B. forficata* on hydrogen peroxide levels in homogenates of adult flies hatched from larvae fed on high sucrose diets. **(A)** probe alone, **(B)** control, **(C)** HSD + *S. cumini*, **(D)** HSD + *B. forficata,* **(E)** HSD. Lines correspond to a randomly representative graphic. Data are presented as arbitrary fluorescence units (AFU)±S.E.M by Two-way ANOVA followed by Duncan Test. *$p<0.05$ indicates statistical difference from control; $^{\#}p<0.05$ indicates statistical difference from respective HSD.

Figure 7. Effect of *S. cumini* and *B. forficata* extracts on SOD **(A)**, CAT **(B)**, and GST **(C)** enzymes activities of 1-3 days old adult flies grown on HSD. Data are presented as mean±S.E.M by Two-way ANOVA followed by Duncan Test. *$p<0.05$ indicates statistical difference from control; $^{\#}p<0.05$ indicates statistical difference from respective HSD.

Figure 8. Effect of *S. cumini* and *B. forficata* on AChe **(A)** and δ-ALA-D **(B)** activities in flies hatched from larvae fed on high sucrose diets. Data are presented as mean±S.E.M by Two-way ANOVA followed by Duncan Test. *$p<0.05$ indicates statistical difference from control; $^{\#}p<0.05$ indicates statistical difference from respective HSD.

7. Figures

Figure 1

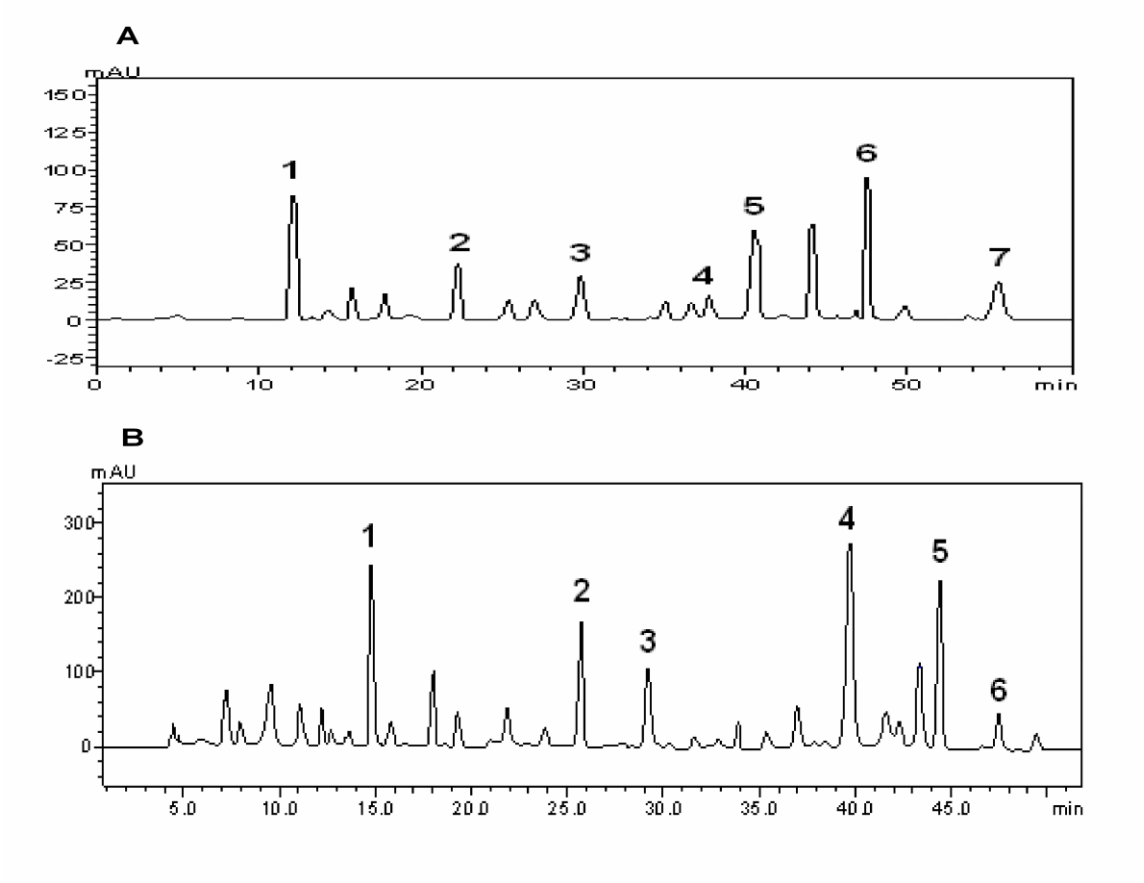


Figure 2

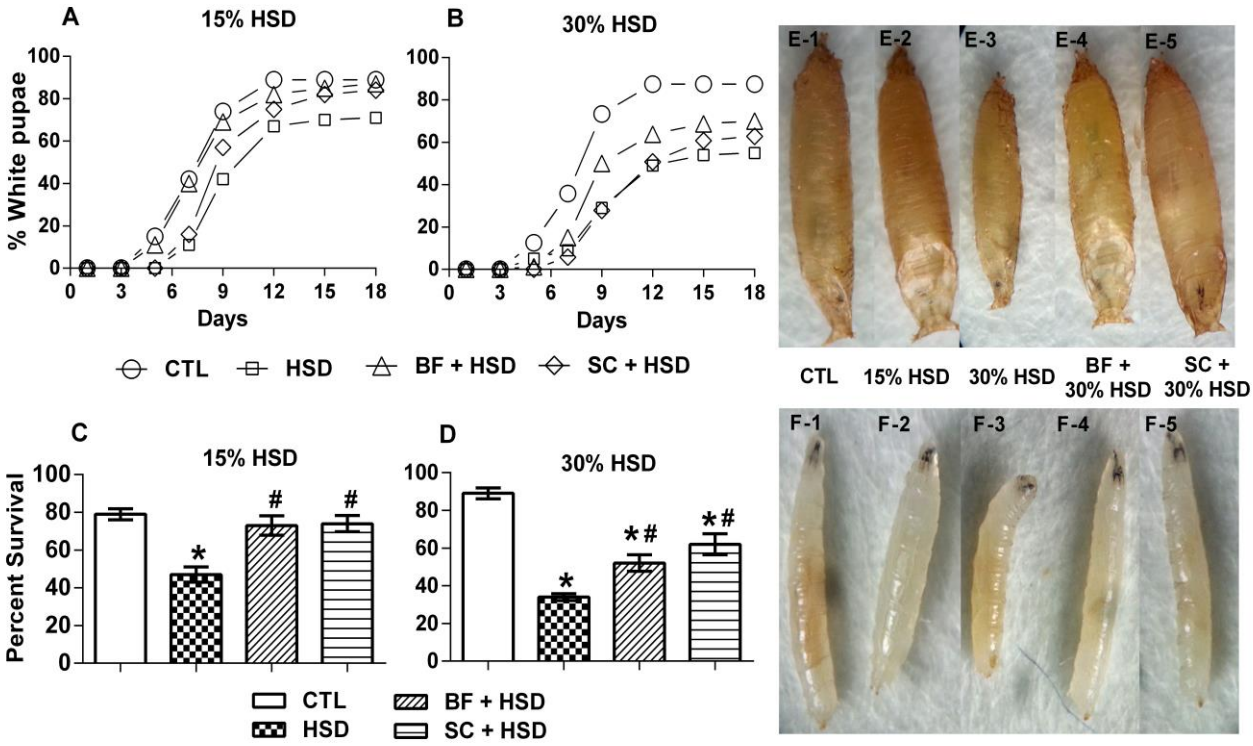


Figure 3

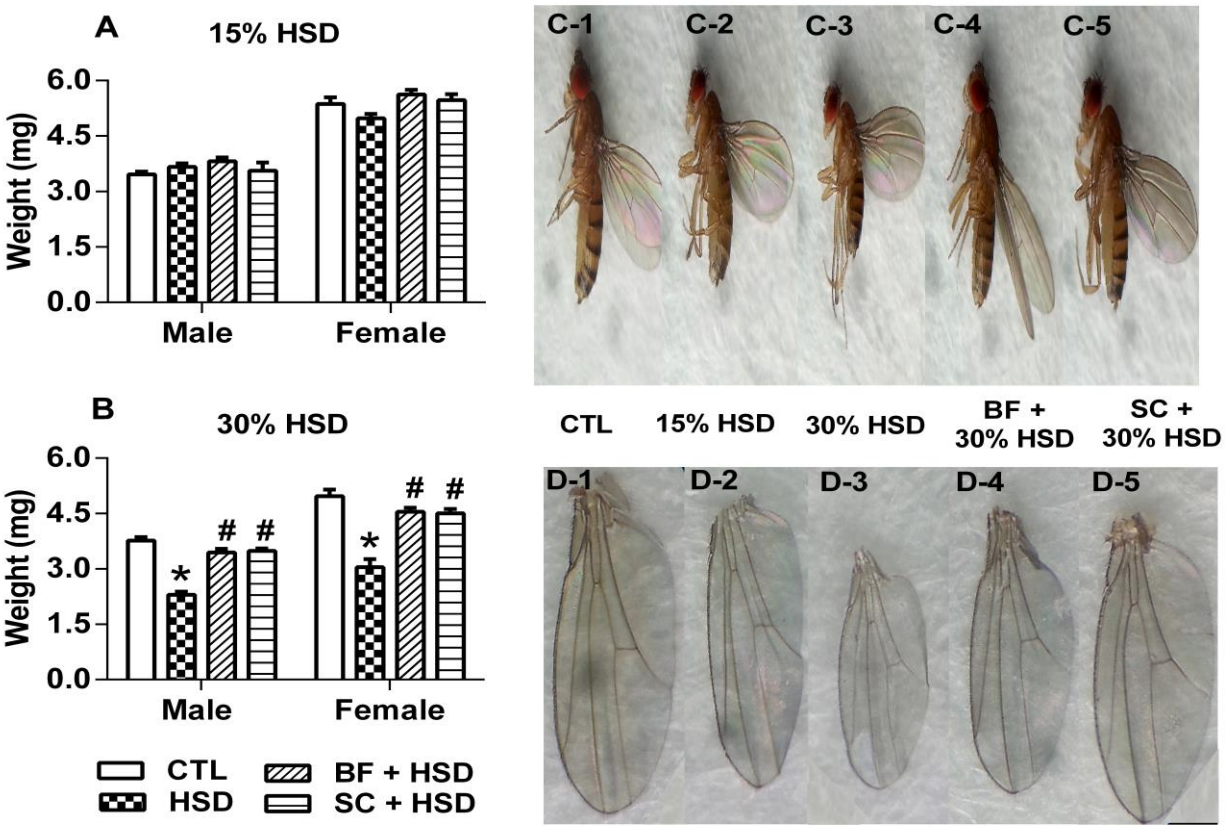


Figure 4

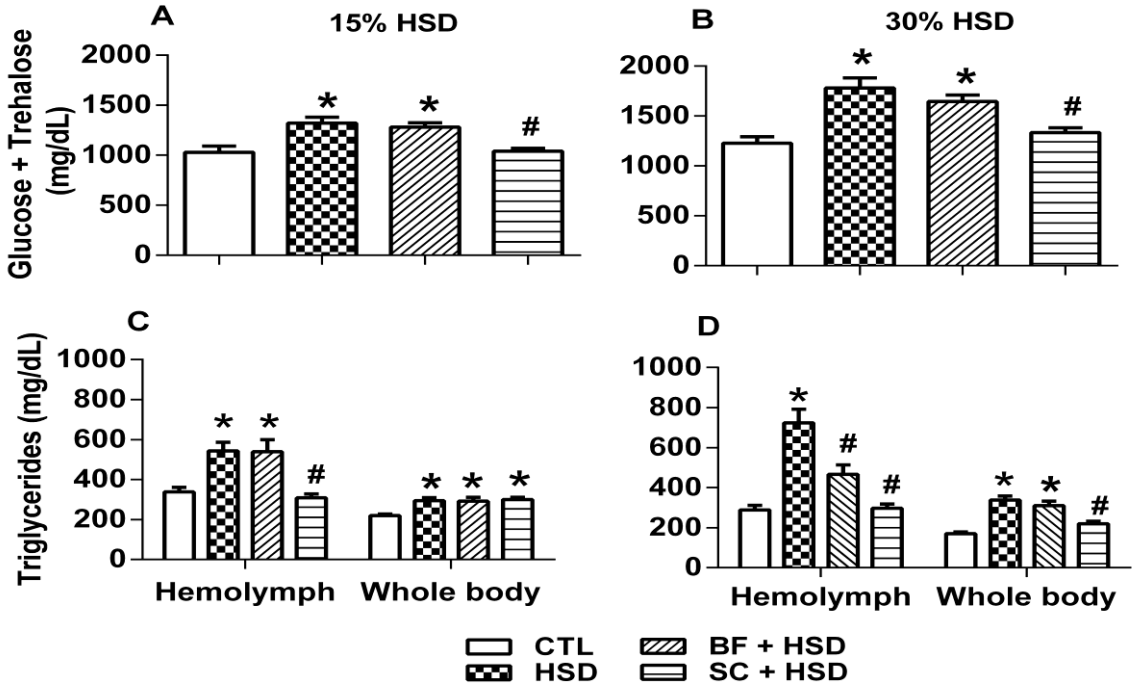


Figure 5

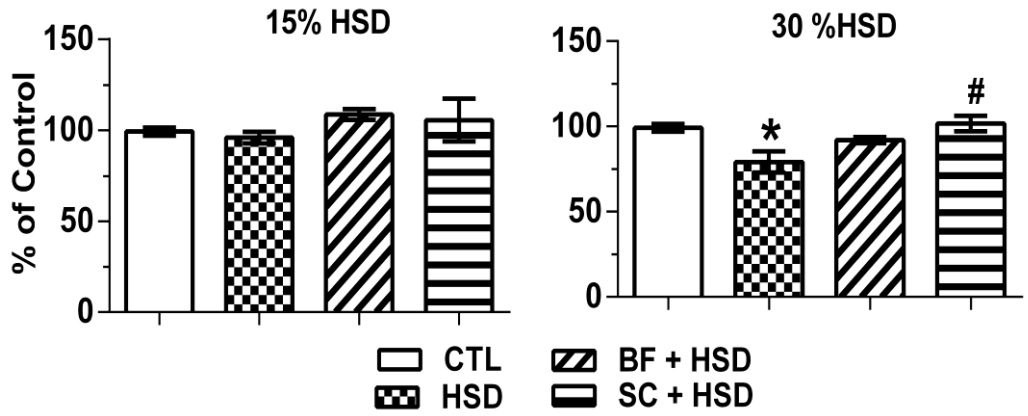


Figure 6

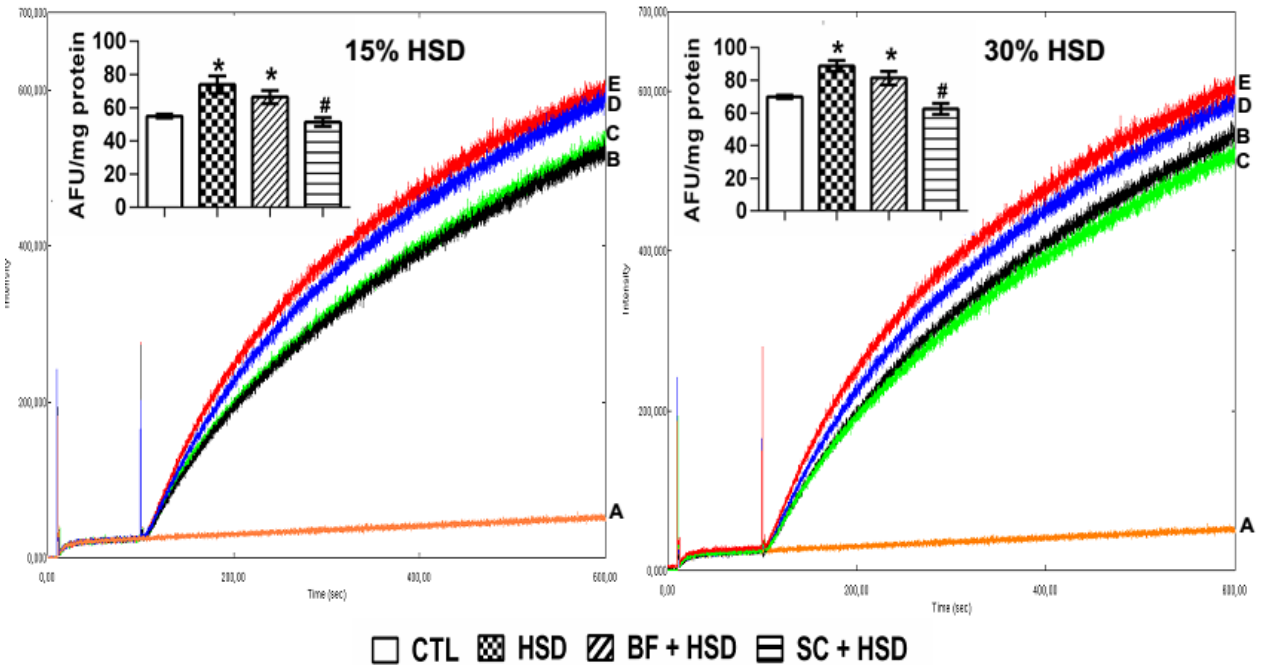


Figure 7

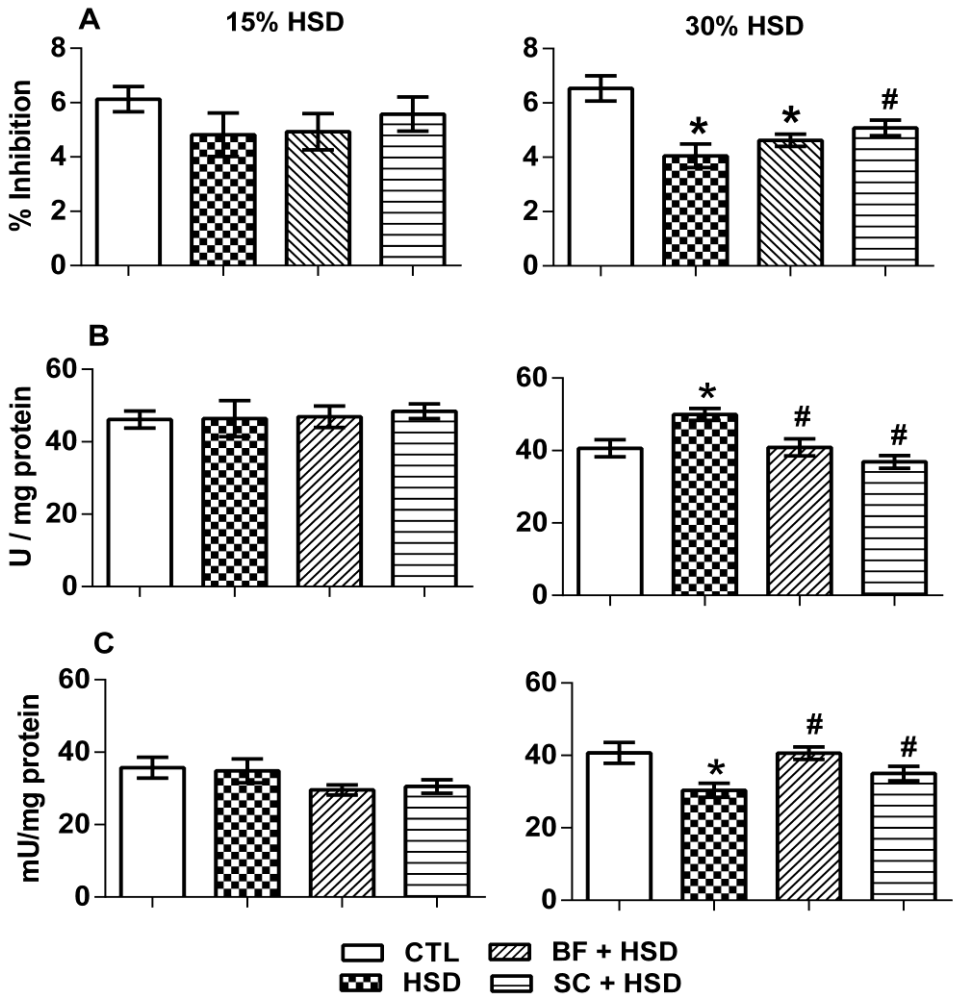
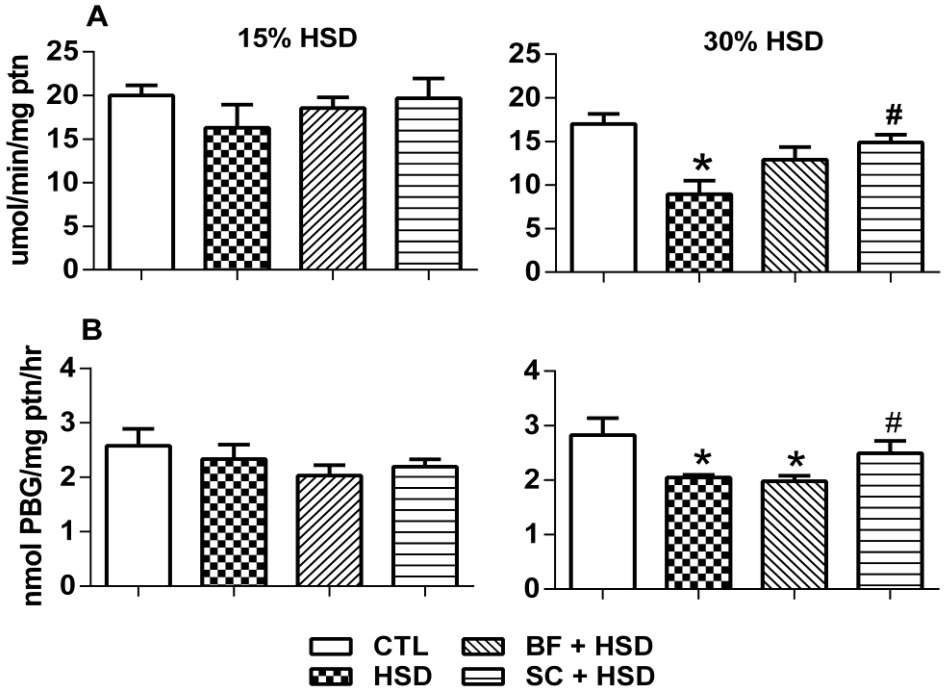


Figure 8



4. CONCLUSÕES

4.1 Conclusões Específicas

- O consumo de ambas as dietas ricas em sacarose atrasou nas larvas o tempo para o surgimento da pupa, reduziu o número de pupas brancas e aumentou a taxa de mortalidade das moscas; parâmetros desenvolvimentais associados à sinalização da insulina;

- Ambas as dietas promoveram um aumento significativo nos níveis de glicose+trealose e triglicerídeos (hemolinfa e corpo total), características metabólicas indicativas de disfunção na cascata de reações mediada por insulina e sintomas do DM tipo 2;

- A dieta com teor mais alto de sacarose reduziu o peso corporal de machos e fêmeas adultos alimentados durante o período larval, características associadas ao desenvolvimento de sintomas do DM tipo 2;

-A dieta rica em sacarose 30% alterou a atividade das enzimas CAT, SOD, GST, acetilcolinesterase e δ-ALA-D, bem como diminui a viabilidade mitocondrial e aumentou os os níveis de H_2O_2 no homogenato de moscas adultas nascidas de larvas que receberam a dieta. Esses dados indicam o desenvolvimento de estresse oxidativo, o qual pode estar associado com a hiperglicemia gerada pelo consumo excessivo de açúcar;

- A suplementação com os extratos de *S. cumini* e *B. forficata* foi efetiva em reduzir a maioria das alterações causadas pelo consumo de ambas dietas ricas em sacarose, destacando-se na maioria dos parâmetros analisados o efeito benéfico da planta *S. cumini*. É plausível supor que a ação antioxidante dos extratos esteja envolvida em tais efeitos, uma vez que os marcadores de danos oxidativos aumentados pelo consumo das dietas foram reduzidos pela suplementação com os extratos. Além disso, o efeito dos extratos sobre os parâmetros desenvolvimentais avaliados, sugerem que as plantas *S. cumini* e *B. forficata* também possam modular vias de sinalização reguladas pela insulina, as quais requerem estudos posteriores.

4.2 Conclusão Geral

Este estudo mostrou que o excesso de açúcar na dieta induziu alterações fisiológicas significativas na mosca da fruta *D. melanogaster*, que atingiram tanto a fase larval como a fase adulta. As modificações observadas abrangeram parâmetros de desenvolvimento, metabólicos e antioxidantes, as quais podem estar associadas com modificações na via de sinalização da insulina e com o estresse oxidativo provocado pela hiperglicemia. Os resultados deste trabalho também reúnem dados que mostram o efeito benéfico do uso dos extratos das plantas *S. cumini* e *B. forficata* nessas condições, reduzindo efetivamente a maioria dos efeitos deletérios causados pelo consumo de sacarose.

De forma geral, conclui-se que a *Drosophila melanogaster* constitui um modelo efetivo para se estudar patologias humanas associadas com a composição da dieta e destacam principalmente a planta *S. cumini* como um promissor candidato em estudos voltados para a terapêutica de desordens metabólicas, como o DM tipo 2.

5. PERSPECTIVAS

Realização de estudos para avaliar se o efeito protetor dos extratos poderia estar associado com a modulação de vias de sinalização da insulina: avaliação da expressão de alguns genes envolvidos na cascata de sinalização de insulina em *Drosophila melanogaster*: Dilps - 2, 3 e 5, InR, Chico, FOXO, AKT, PTEN, TORC, SIK2, Impl2;

Uma vez que os extratos reduziram alguns marcadores bioquímicos de estresse oxidativo, efetuar a análise da expressão de alguns genes envolvidos nas respostas do sistema antioxidante: SOD, CAT, proteínas de choque térmico/Heat Shock proteins HSP27, HSP70, HSP83 e o fator de transcrição NRF2 em moscas.

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