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High-sucrose diet induces diabetic-like phenotypes and oxidative stress in *Drosophila melanogaster*: Protective role of *Syzygium cumini* and *Bauhinia forficata*

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ABSTRACT

Diet is a key component for development and longevity of organisms. Here, the fruit fly was used to evaluate the detrimental effects caused by consumption of high-sucrose diets (HSD), namely phenotypic responses linked to insulin signaling and oxidative stress. The protective effects of extracts from medicinal plants *Syzygium cumini* and *Bauhinia forficata* were investigated. HSD intake (15% and 30%) delayed the time to pupation and reduced the number of white pupae. In adult flies, the intake of diets was associated with mortality and increased levels of glucose + trehalose, triacylglycerols and hydrogen peroxide. Indeed, 30% HSD induced body-weight loss, mitochondrial dysfunction and changes in acetylcholinesterase, δ-aminolevulinate dehydratase and antioxidant enzymes activity. Catalase, superoxide dismutase, keap1, HSP70, dILP-5 and Insulin receptor mRNA levels were over-expressed in flies emerged from 30% HSD. The extract treatments blunted the developmental alterations elicited by diets. *Syzygium cumini* extract was more efficient than *B. forficata* in reducing hyperglycaemia, redox disturbances and the changes in mRNA expression of insulin receptor.

1. Introduction

It is known that dietary caloric excess shortens lifespan and leads to metabolic derangement in a wide array of species, including humans [1,2]. Epidemiological studies have shown that the diet components are the most important environmental risk factors for the development of chronic metabolic diseases [3,4]. In this scenario, a special attention has been given to carbohydrate since high-sugar diets have long been linked to metabolic problems such as obesity and insulin resistance, which are important hallmarks of type 2 diabetes mellitus (DM) [5]. According to the World Health Organization (WHO), the prevalence of type 2 DM has increased dramatically worldwide and will affect about 400 million adults in 2030 [5].

The mechanisms underlying the long term complications of type 2 DM are still not completely understood. However, accumulating evidence strongly points to the hyperglycaemia as a potent inducer of cell damage via oxidative stress [6,7]. In fact, biochemical pathways activated under hyperglycaemic condition such as glucose auto-oxidation, protein glycation and advanced glycation end products (AGEs) formation can trigger oxidative

Abreviations: D. melanogaster, *Drosophila melanogaster*; HSD, high sucrose diet; DM, diabetes mellitus; SC, syzygium cumini; BF, *Bauhinia forficata*; IPC, insulin-producing cells; dILP, insulin-like peptides; InR, insulin receptor; AGEs, glycation end products; HPLC, high performance liquid chromatography; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CAT, Catalase; SOD, superoxide dismutase; GST, glutathione S-transferase; GSH, reduced glutathione; HSP, heat shock protein; δ-ALA-D, delta-aminolevulinate dehydratase; PBG, porphobilinogen; H_2O, $_{22}$hydrogen peroxide; TGs, triacylglycerols; TMED, (*N*,*N*,*N*′,*N*′ tetrametiletilenediamine); EDTA, ethylenediamine tetraacetic acid; Nrf2, nuclear factor E2-related factor-2; DMSO, dimethyl sulphoxide; Nipagin, methyl-p-hydroxybenzoate; AChe, acetylcholinesterase; q-PCR, quantitative real-time PCR.

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damage via free radical overproduction and antioxidant defenses impairment [6–10].

Currently, various studies have reported the potential of functional/nutraceutical foods in reducing the risk of age-related decline and chronic diseases, including DM and its complications [11,12]. In this field, medicinal plants are well-known as an excellent source of bioactive non-nutrient compounds with potential health effects. The phytochemicals and extracts derived from plants have been highlighted in numerous studies as effective agents in the management of oxidative events associated with various ailments [12–14].

The medicinal plants *Bauhinia forficata* Link (*B. forficata*, Leguminosae) known as "cow's foot" ("pata-de-vaca") and *Syzygium cumini* (L) Skeels (*S. cumini*, Myrtaceae syn: Eugenia jambolana) known as Jambolan or Java plum ("Jambolão"), are popularly used as hypoglycaemic agents in folk medicine [15]. As infusion and decoction, the leaves from plants are commonly used by population not exclusively to treat diabetes complications, but as regular tea [16]. Thus, the ethnopharmacological use of the leaves from *S. cumini* and *B. forficata* has popularized their consumption as common teas or as food supplement to promote health by preventing chronic diseases such as DM [16–20]. Accordingly, in Brazil, there are large and medium companies commercializing the tea from *B. forficata* and/or tea formulations containing it that are found in almost all supermarkets. The commercial use of leaves from *S. cumini* is much more limited than that of *B. forticata*, but its commercialization is growing considerably in Brazil. Though consumed as nutraceuticals, the molecular and biochemical processes underlying the pharmacological and/or toxicological properties of leaves from both species are still unclear [15].

In recent years, the fly *Drosophila melanogaster* (*D. melanogaster*) has emerged as an advantageous alternative organism to mammalian models for exploring different human pathologies, including metabolism-related disorders as obesity and DM [21,22]. Many biochemical mechanisms involved in the control of growth and metabolic processes in humans are present in the fly [23]. Of particular importance, neuroendocrine architecture and mechanisms in *D. melanogaster* resemble those found in mammals [24]. For instance, *D. melanogaster* has insulin-producing cells (IPC), insulin-like peptides (dILPs) and receptor (InR); conserving the molecular insulin/insulin-like growth factor signaling pathways [25]. In this sense, literature data have demonstrated that high sugar diet elicits insulin-resistant phenotypes in *D. melanogaster* that represent the pathophysiology of type 2 DM in humans [22,26]. These phenotypes are normally characterized by elevated fat deposition and circulating glucose, systemic insulin resistance, shortened fecundity and life-span in adult flies [22,25,26,27]. In larvae, insulin signaling cascade is involved in metabolic homeostasis and growth. 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 [22,27]. Consequently, *D. melanogaster* represents an attractive organism model for studying diet-induced metabolic disorders and potential therapeutic strategies to remedy them.

Considering the negative impact of diet rich in carbohydrates on developmental/metabolic homeostasis, here we used *D. melanogaster* for exploring the effects of high-sucrose consumption on phenotypic responses consistent with type 2 DM and oxidative events. In larvae fed on high-sucrose diet were evaluated mainly characteristics linked to insulin signaling as longevity and growth. In adult flies, some antioxidant/oxidant parameters were assayed as endpoint markers of toxicity. Simultaneously, hypoglycemic plants were tested as therapeutic strategies.

2. Material and methods

2.1. 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). DNase I, TRIZOL reagent and DNase-free RNase were purchased from Invitrogen. iScrip cDNA Synthesis Kit was purchased from Bio-rad. Acetic acid, gallic acid, chlorogenic acid and caffeic acid were purchased from Merck (Darmstadt, Germany).

2.2. Leaf extracts preparation

Bauhinia forficata and *Syzygium cumini* leaves were collected in the Jardim Botânico of the Universidade Federal de Santa Maria (UFSM) in Santa Maria-RS, Brazil. The material was identified and authenticated in the herbarium of the Institution. The plant leaves were dried (5% humidity, room temperature), powdered (150–300 μm) and then prepared as infusion at concentration of 30% (30 g of powder/100 ml of water) for 30 min, which is similar to the folk-medicine preparation method. Afterwards, the samples were

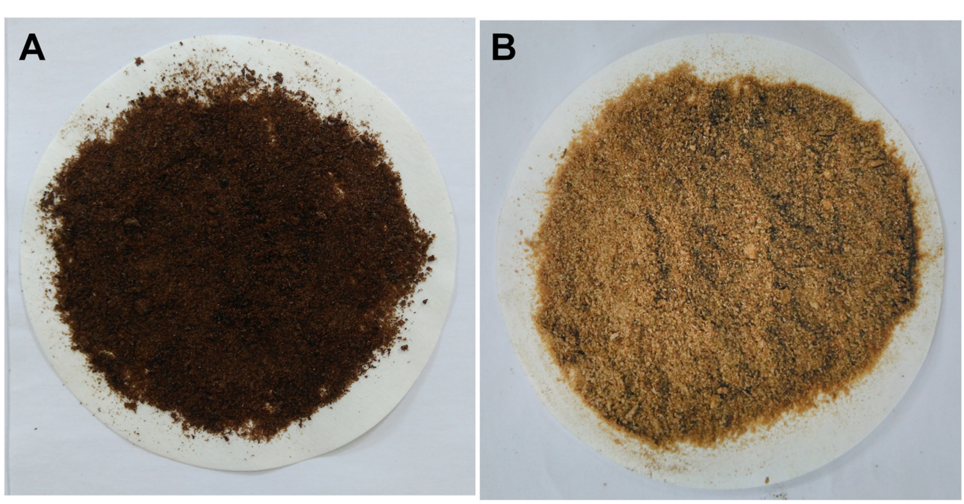

Fig. 1. Images of (A) *Bauhinia forficata* and (B) *Syzygium cumini* lyophilized extracts. Leaves from plants were dried, powdered and extracted with distilled water. Afterwards, the samples were filtrated and subsequently lyophilized to obtain the final powder.