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Original article

Treatment with tucumã oil (*Astrocaryum vulgare*) for diabetic mice prevents changes in seric enzymes of the purinergic system: Improvement of immune system

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ABSTRACT

The aim of this study was to evaluate the enzymatic activity of the purinergic system in sera samples from alloxan-induced diabetic mice treated with tucumã oil (*Astrocaryum vulgare*). For this, the mice were divided into four groups (n = 6): control/water (the group CW); control/tucumã oil (the group CT); diabetic/water (the group DW), and diabetic/tucumã oil (the group DT) treated for 14 days with 5.0 mL kg^{-1} via oral gavage. On day 14 post-treatment, mice were submitted to euthanasia and blood samples were collected by cardiac puncture. Tucumã oil treatment significantly decreased ($p < 0.05$) blood glucose levels in the group DT compared to the group DW. These results demonstrated an increase ($p < 0.05$) in NTPDase (adenosine triphosphate (ATP substrate) or adenosine diphosphate (ADP substrate)), 5′-nucleotidase (AMP substrate) and adenosine deaminase (ADA; adenosine substrate) activities in serum from the group DW compared to the group CW. Tucumã oil treatment prevented these alterations in the group DT compared to the group DW, and restored these parameters near to the group CW. In summary, the treatment with tucumã oil was able to modulate the alterations caused by hyperglycemia probably by the presence of carotenoids compounds, maintaining normal levels of ATP, ADP, AMP and adenosine, molecules that could exhibit anti-inflammatory properties, depending on their concentration. Thus, the tucumã oil is a promising natural compound with protective action against diabetes and its side effects, such as changes in the purinergic system, improving the immune system.
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1. Introduction

Diabetes mellitus (DM) is a chronic disorder of carbohydrate, fat and protein metabolism characterized by hyperglycemia resulting from defects in insulin secretion, action or both [1], and it is considered the most significant devastating disease and causative of death in modern society. According to Gregg et al. [2], DM is considered a healthcare problem in the United States, affecting 29 million patients in 2014 and incurring yearly costs that exceed $245 billion, affecting 8.3% of adult population worldwide. Low-grade inflammation has been linked with many mechanisms in the pathogenesis of DM, and has been identified as one of the risk factors for the disease [3], as well as associated with microvascular complications, such as nephropathy, retinopathy and neuropathy [4,5], and the investigation of systems related to inflammation, such as enzymes of the purinergic system, can contribute to a better understanding of the pathophysiology of DM [6].

The purinergic system has many important functions in the body, including regulation of immune response. This event occurs due to interactions of nucleotides with purinoreceptors present in the plasma membrane of innumerable cells [7]. Physiological extracellular ATP levels have important functions in physiological conditions [8], however excessive ATP levels exerts a pro-inflammatory role during many diseases, while adenosine (Ado) is an endogenous purine nucleoside that acts as an endogenous regulator of immunity, protecting the host tissue from damage and plays an important role in lymphocyte differentiation and proliferation [9,10]. The importance of these nucleotides is associated with the essential function of the enzymes that degrades nucleotides and promotes its suitable concentration

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extracellularly. The nucleotide enzymatic regulation initiates with NTPDase that hydrolyses ATP into ADP and AMP. Degradation continues with 5′-nucleotidase activity that hydrolyses AMP into Ado, while ADA hydrolyses Ado into inosine [11]. Studies have demonstrated that DM alters the ectoenzymes of purinergic system on brain and platelets of alloxan-induced diabetic mice and rats [12–14]. Recently, a study conducted by Capiotti et al. [15] has demonstrated that alterations on E-NTPDase, E-5'nucleotidase and E-ADA activities contribute directly to the neuropathology of DM.

Astrocaryum vulgare, commonly called tucumã in Brazil, belongs to the Arecaceae family and grows in the Northeast region of Brazil, and it is mainly used in human nutrition due to its high carotenoid content in the pulp oil, that has hypoglycemic effect [16]. A study conducted by Bony et al. [17] demonstrated the anti-inflammatory properties of tucumã oil against J774 macrophages in vitro and in mice model of endotoxin shock, as well as acute and chronic model of pulmonary inflammation [18] According to these authors, the anti-inflammatory properties is probably mediated by fatty acid and the presence of antioxidant compounds such as carotenoids, and anti-inflammatory molecules like sterols. In this context, considering the role of inflammation on pathogenesis of DM, the anti-inflammatory effect of tucumã oil, and the importance of NTPDase, 5′-nucleotidase and ADA on the inflammatory immune response, the aim of this study was to evaluate the activity of these enzymes in sera samples of alloxan-induced diabetic mice treated with tucumã oil.

2. Materials and methods

2.1. Chemicals

The chemicals (except tucumã oil) used in the experiment were obtained from Sigma Chemical Co. (St. Luis, MO, USA). Tucumã oil (*A. vulgare*) was purchased from Amazon Oil Industry (Amazonia, Brazil), extracted from pulp fruit by the cold pressing method.

2.2. Tucumã oil characterization

Tucumã oil was previously characterized by gas chromatograph accoupled to flame ionization detector (GC-FID), as recently published in details by Baldissera et al. [16]. Twenty fatty acids were identified in tucumã oil, being the oleic/elaidic acid (368.7 mg/g of oil) the most abundant compound [16].

2.3. Animals

Adult female Swiss mice (70 days; 28 ± 1.5 g) were used in this experiment. All the animals were acclimated for two weeks prior to the commencement of the experiment. Animals were housed in polyurethane cages under hygienic conditions and maintained in air conditioned room (22 ± 1 °C) with a 12 h light/dark cycle. Standard pellet diet and water were provided *ad libitum* as approved by the Ethics Committee for Use of Animals (CEUA) of Universidade do Estado de Santa Catarina (UDESC), under protocol number 8055270416.

2.4. Induction of experimental diabetes

Experimental diabetes was induced in overnight fasted (12 h fasted) mice by a single intraperitoneal injection of alloxan monohydrate (75 mg kg^{-1}) dissolved in sterile cold saline solution (0.9%), as previously published by Baldissera et al. [16]. Age-matched control mice received an equivalent amount of saline solution. Alloxan monohydrate-treated mice received 5% of glucose in the water for 24 h after diabetes induction in order to reduce death due to hypoglycemic shock. After 72 h of alloxan monohydrate administration, diabetes was confirmed by measuring the fasting blood glucose levels by performing a small puncture in the tail vein. Glucose levels were measured with a portable glucometer (Advantage, Boehringer Mannheim, MO, USA). Only animals with fasting glycemia over 250 mg/dL were considered diabetic and used for the present study. The result of blood glucose levels has previously published by Baldissera et al. [16] proving the diabetic condition.

2.5. Study design

A total of 24 mice (12 normal and 12 diabetic) were randomly divided into four groups (six mice per group): control/water (the group CW); control/tucumã oil (the group CT); diabetic/water (the group DW) and diabetic/tucumã oil (the group DT). The animals belonging to the groups CT and DT received 5 mL kg^{-1} of tucumã oil, while the groups CW and DW received water at the same dose (used as control). Tucumã was administered orally between 10 and 11 AM once a day for 14 days. The treatment was started on the 4th day after alloxan monohydrate injection, i.e., one day after diabetes confirmation, which was considered the first day of treatment, based on Baldissera et al. [16].

The dose choice of 5 mL kg^{-1} of tucumã was based on a previous study published by Baldissera et al. [16] that shown hypoglycemic effect against type 1 diabetes. Oral route was used since it represents how people would use tucumã oil. The groups CW and DW received water to suffer the same procedure of treated tucumã oil groups (CT and DT).

2.6. Sampling

On day 14 post-treatment, mice were anesthetized with isoflurane for blood collection (1 mL per mice) by cardiac puncture. Blood was allocated in tubes without anticoagulant, and serum was obtained from blood samples after centrifugation at 3500 × *g* for 10 min at 4 °C, and stored at −20 °C. Serum was used to determine the activities of enzymes of the purinergic system (NTPDase, 5′-nucleotidase and ADA). Protein was measured by the Coomassie Blue method according to Bradford [19] using serum albumin as standard.

2.7. Seric NTPDase and 5′-nucleotidase activities

NTPDase and 5′-nucleotifase activities in sera samples were determined as previously described by Oses et al. [20]. The reaction mixture for the NTPDase activity contained 3 mM of ATP or ADP as substrate and 112.5 mM Tris-HCl (pH 8.0). The reaction mixture for 5′-nucleotidase was composed of 3 mM of AMP as substrate and 100 mM Tris-HCl (pH 7.5). The reaction mixtures were incubated with approximately 1.0 mg of protein at 37 °C for 40 min on a final volume of 0.2 mL. The reaction was stopped by the addition of 0.2 mL of 10% trichloroacetic acid (TCA). All samples were centrifuged at 5000 × *g* for 5 min to eliminated precipitated protein and the supernatant was used for the colorimetric assay. The samples were chilled on ice for 10 min. Released inorganic phosphate (Pi) was assayed by the method of Chan et al. [21] using malachite green as the colorimetric reagent, and KH_2PO_4 as standard. Enzyme activities were expressed as nanomoles of Pi released per min per milligram of protein (nm of Pi/min/mg of protein).

2.8. Seric ADA activity

The determination of seric ADA activity was performed as described by Giusti and Gakis [22], which is based on the direct measurement of ammonia formation, produced when ADA acts in

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