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Brazilian sunberry (*Solanum oocarpum* Sendtn): Alkaloid composition and improvement of mitochondrial functionality and insulin secretion of INS-1E cells

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ABSTRACT

Chronic high-glucose levels induce the generation of reactive oxygen species leading to mitochondrial dysfunction, which is one of the pathological triggers in the development of diabetes. This study investigated the alkaloid composition of two fruits of the genus *Solanum*, fruta-do-lobo (*Solanum lycocarpum*) and juá-açu (*Solanum oocarpum*), and their capacity to protect against oxidative damage and defective insulin secretion induced by chronic high-glucose levels. LC-MS and molecular network of fruit crude extracts reveals that juá-açu and fruta-do-lobo contain kukoamines and glycoalkaloids, respectively. Two purification processes were used to enrich those alkaloids. Fruta-do-lobo extract rich in glycoalkaloids showed a strong cytotoxicity effect, however the juá-açu enriched extract was able to protect mitochondrial functionality against glucotoxicity and stimulate insulin secretion even under conditions of hyperglycemia. These results are promising and suggest that juá-açu is a potential source of bioactive compounds for adjuvant/co-adjuvant therapy for diabetes.

1. Introduction

In recent years, the interest in finding natural antioxidant compounds with effective biological activities to control and treat diseases (such as antidiabetic) has increased (Li, Lin, Chen, Xie, & Chen, 2018). One prominent plant family often associated to molecules with antidiabetic and antioxidant effects is the Solanaceae family, especially the *Solanum* genus, such as *Solanum melongena* (Kwon, Apostolidis, & Shetty, 2008), *Solanum americanum* Mill (Silva, Almeida-Lafetá, Borges, & Staerk, 2017), *Solanum torvum* Swartz (Gandhi, Ignacimuthu, Paulraj, & Sasikumar, 2011) and *Solanum anguivi* (Elekofehinti, Kamdem, Kade, Rocha, & Adanlawo, 2013).

Diabetes mellitus represents a group of autoimmune, metabolic and genetic disorders, characterized by hyperglycemia, micro and macrovascular complications (Egan & Dinneen, 2019; Ighodaro, 2018). It can also be divided in type 1 and type 2 (TD2), being TD2 the most common, accounting for approximately 90 % of all diabetes cases (International Diabetes Federation, 2019). In chronic hyperglycemic conditions, the production of reactive oxygen species (ROS) becomes exacerbated, so that antioxidant defense systems are not able to neutralize them, which further intensifies the oxidative stress (Ighodaro, 2018). The oxidative stress has been associated with disease complications by impairing major glucose regulatory mechanisms in TD2, especially due to insulin resistance in target tissues and pancreatic islet malfunction (Asmat, Abad, & Ismail, 2016).

The genus *Solanum* is strongly characterized by the presence of glycoalkaloids, especially steroid saponins (*e.g.*, solasonine and solamargine), which were associated to antidiabetic properties (Al Sinani & Eltayeb, 2017; Pereira et al., 2021) and dihydrocaffeic acid derivatives of polyamines (*e.g.*, kukoamines, phenolamides), which were shown to attenuate oxidative stress and insulin resistance (Hu et al., 2015; Li, Zhou, Chen, Zhang, & Wang, 2017; Wang, Snooks, & Sang, 2020; Zhang et al., 2016).

Therefore, natural sources of compounds that may act in diabetes

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control are welcome, especially considering the non-exploited potential of fruits from Cerrado Brazilian biome such as *Solanum* genus. Hence, in this study we investigated the alkaloid composition from two fruits of the genus *Solanum*, fruta-do-lobo (*Solanum lycocarpum* St. Hill) and juá-açu (*Solanum oocarpum* Sendt.), and the capacity of those compounds to protect against oxidative damage, cell death, and defective insulin secretion induced by chronic high-glucose levels.

2. Material and methods

2.1. Sample acquisition

Juá-açu fully ripe (Fig. 1, A) was collected in October 2017 in Campina do Monte Alegre (S 20.555.209; W46.145.379) located in the state of São Paulo, Brazil. Fruta-do-lobo (Fig. 1, B) was collected in June 2017 in Carmo do Rio Claro (S 20.555.209; W46.145.379), located in the state of Minas Gerais. Specimens were identified by Dr. Ingrid Koch and Dr. Leandro Giacomini, and voucher specimens were deposited at the herbarium of the University of Campinas (Unicamp) (UEC 197730 and 197248, respectively). The fruits were manually washed, the peel

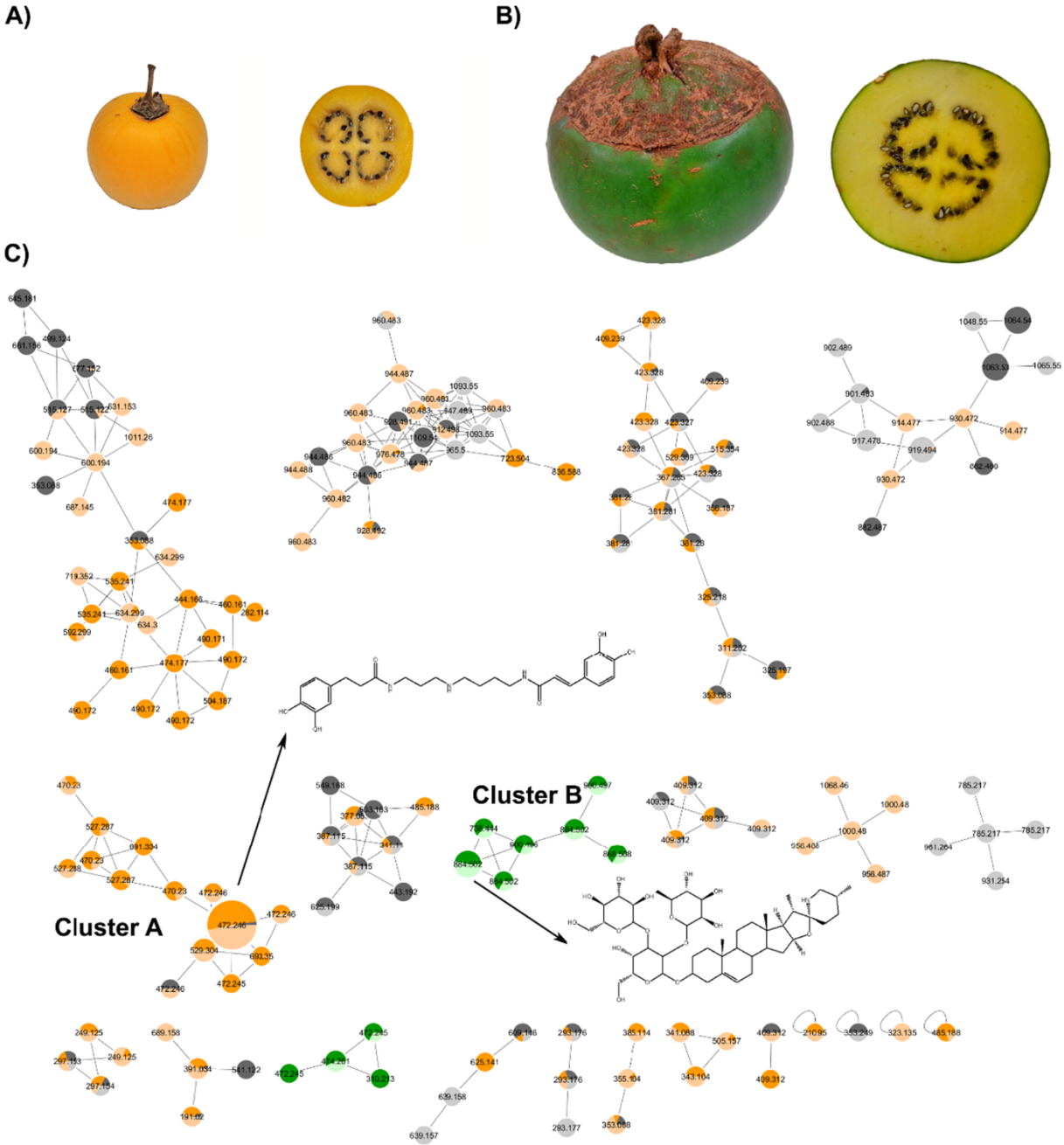

Fig. 1. A) *Solanum oocarpum* Sendt riped, B) *Solanum Lycocarpum* St. Hill riped, C) Molecular Network of fruit extracts. Each detected compound is represented by a node, which are colored according to their sources: dark green – FL peel; light green – FL – pulp; dark grey – FL seed; dark orange – JA peel; light orange – JA pulp and light grey – JA seed. The node size is related to the sum of precursor intensity, i.e., nodes sizes correspond to relative compound abundance. (https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=e6cb338f3edd425dbfd5ccf4922faeac). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was carefully separated from the pulp using a sharp knife and the seeds were removed.

2.2. Extract preparation

The remaining pulp was freeze-dried (Liotop, Liobras, Brazil) for 48 h, packed (200B Selovac, São Paulo, Brazil) under vacuum in low density polyethylene bags, and frozen at −18 °C until milling, which was carried out in a blender (Colombo AR 2 L, Itajobi, Brazil). Extracts were prepared according to Pereira et al. (2018), using ethanol:water (70:30/v:v) in a concentration of 100 mg/mL (dried fruit/solvent). The resulting extracts (fruta-do-lobo crude extract, FLC, and juá-açu crude extract, JAC) were concentrated under vacuum until they were completely dry and were resuspended in ultrapure water to return to the original concentration of 100 mg/mL (dried fruit/solvent).

2.2.1. Juá-açu alkaloids enrichment

The isolation of the alkaloids was performed via solid phase extraction using a polyamide resin as an adsorbent according to Cheung, Li, and Di (2015), with modifications. Initially, a crude extract was prepared, where 100 g of freeze-dried juá-açu pulp was mixed with 100 mL of a ethanol:water (50:50/v:v) solution in a homogenizer (Ultra-Turrax T-25, Germany) for 2 min and sonicated for 1 h. After that, the solution was centrifuged (5 °C, 15 min, 8,944 × g) (Rotanta 460R, Hettich, Germany) and the supernatant was reserved. The residue was again subjected to extraction and this procedure was repeated 3 times. The supernatants were homogenized and concentrated under vacuum (R-II Rotary Evaporator, Bucchi, Switzerland) until they were completely dried, yielding about 10 g of a crude extract. Five grams of crude extract was mixed with 200 mL of water to give an aqueous solution of the crude extract of juá-açu. Five grams of polyamide (Discovery DPA-6S, Supelco, USA), as adsorbent immobile phase, was added into the aqueous solution of the crude extract of kukoamine. After mixing for 2 h at room temperature using a magnetic stirrer, the resultant mixture was filtered under suction to obtain kukoamine-adsorbed polyamide. The remaining filtrate was repeatedly adsorbed with polyamide 3 times using 5 g of polyamide per time. The combined resins were suspended in 100 mL of water filtered and suspended again in 250 mL of a methanol:water (50:50/v:v) solution, follow by filtration and rinsing with the same solution to remove impurities. Then, the polyamide was suspended in 50 mL of 0.5 % aqueous acetic acid solution and were stirred continuously to desorb the alkaloids. The solution obtained after desorption was filtered through a microporous filter membrane (0.22 μm) and the solvent was evaporated resulting in 10 mg of juá-açu enriched extract (JAE). The extract was resuspended in 0.754 mL of DMSO resulting in a 20 mM solution which was used in the biological assays (considering a molar mass of 679u as major constituent of JAE).

2.2.2. Fruta-do-lobo glycoalkaloids enrichment

A crude extract was prepared by mixing 200 g of freeze-dried fruta-do-lobo pulp with 500 mL of ethanol:water (70:30/v:v), which was left standing overnight at room temperature. After that, the plant material was removed by filtration and mixed with 12 % v/v acetic acid solution until a stable pH of 4 was reached. After 48 h of acid digestion, the mixture was filtered again and both supernatants were combined. The solution was then centrifuged (1431 x *g* for 20 min) to remove insoluble materials and the supernatant was mixed with sodium hydroxide solution (10 %, v/v) until a pH of 10 was reached. The supernatant was then extracted twice with ethyl acetate (300 mL) and then concentrated in a rotary evaporator until complete removal of the solvent. The obtained solution was filtered through a microporous filter membrane (0.22 μm) and the solvent was evaporated resulting in an oil of fruta-do-lobo enriched extract (FLE). The extract was resuspended in DMSO to reach the final concentration of 25 mM (considering the molar mass of hidroxy-solamargine as major constituent of FLE).

2.3. Alkaloids profile by LC-MS/MS

The resuspended crude and purified extracts were diluted 10 times by pipetting 100 μL of solution in 900 μL of water for LC-MS/MS analysis. The profiles were obtained using an UHPLC (Hewlett Packard, Agilent Technologies 1290 series) coupled to a Q-ToF iFunnel 6550 mass spectrometer using an electrospray ionization (ESI) source with a Poroshell 120 EC-C18 2.7 μm column (2.1 × 100 mm, Agilent). Mobile phase A was milli-Q water with 0.1 % of formic acid (FA); and mobile phase B was ACN with 0.1 % of FA. A flow rate of 0.45 mL min^{-1} was used following the linear gradient: 0–1 min, 5 % B; 1–10 min, 5 % B to 18 % B; 10–13 min 18 % B to 70 % B; 13–15 min, 70 % B to 100 % B (clean up) and 3 min of post time at 5 % B for column re-equilibration. The mass spectrometer voltages and temperatures were set as: VCap 3000 V; fragmentor voltage at 150 V; OCT 1RF Vpp at 750 V; Gas Temperature at 290 °C; Sheath Gas Temperature at 350 °C; Drying Gas at 12 L min^{-1} and the fragmentations were performed using normalized collision energy (NCE) of 30. Mass spectra were acquired in profile and positive or negative ion mode and the acquisition range was 100–1200 *m/z*. Data were treated using Agilent MassHunter Qualitative Analysis B0.7 software. Compound identification was done using GNPS platform and by manual interpretation of MS/MS pattern spectra.

2.4. Evaluation of biological activities of alkaloid fractions in insulin secreting cells (INS-1E).

2.4.1. INS-1E cell culture

INS-1E cells originally developed by Claes Wollheim (Geneva) were kindly supplied by Professor Decio Eizirik, Center for Diabetes Research, Medical Faculty, Universite Libre de Bruxells (ULB), Belgium. This B cell line model was used in several studies due to their capacity of being glucose responsive at physiological range with substantial insulin content levels and a robust metabolism-secretion coupling (Casimir, Chaffard, & Maechler, 2019; Merglen et al., 2004). INS-1E cells were cultured in a humidified atmosphere containing 5 % CO_2 and maintained in RPMI 1640 medium [11 mM glucose, 5 % FBS, 1 % 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1 % sodium pyruvate, 0.1 % β-mercaptoethanol and 1 % penicillin/streptomycin] until 60 to 80 % confluence.

2.4.2. Cell viability using Hoechst (HO) and propidium iodide (PI) dyes

INS-1E cells (10^4 cells per well) were incubated in a 96-well plate with the glycoalkaloid enriched plant extracts (FLE and JAE) for 24–48 h. The percentages of dead cells were determined after 15 min incubation with DNA-binding dyes Hoechst (HO; Sigma; H33342; 5 μg/ml) and propidium iodide (PI; Invitrogen P1304; 5 μg/ml) (Marroquí et al., 2014). Images were obtained using the FLoid® Cell Imaging Station (Thermo-Fischer, Waltham, MA). HO freely passes the plasma membrane and stains DNA in blue, whereas PI, a highly polar dye that is impermeable to cells with intact membranes, stains the DNA of dead cells in red. The percentage of cell death (PI/HO × 100) was quantified using the Image J software (Image J- Bethesda, MD). The results are expressed as average of three independent assays, using in each assay 3–4 replicates of each condition.

2.4.3. INS-1E treatments and function

Cells were seeded at densities of 100.000 cells/well (24 plate) or 10.000 cells/ well (96 plates). After 48 h of seeding, the medium was changed to: RPMI 11 mM glucose (NG, Normal glucose group); RPMI 11 mM glucose plus 100 uM JAE (NG + JAE group); RPMI with high 30 mM glucose (HG, High glucose group), and RPMI HG plus 100 uM JAE (HG + JAE group). Cells were kept with the experimental mediums (NG, HG, NG + JAE or HG + JAE) for 48 h prior to all experimental protocols. Dimethyl sulfoxide (DMSO, Sigma D8418) was used as vehicle.

Glucose stimulated insulin secretion (GSIS)

After the 48 h incubation period with the extracts, INS-1E cells were

incubated for 1 h in glucose-free RPMI GlutaMAXI medium and then incubated for 30 min in Krebs bicarbonate buffer [120 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl2, 1.2 mM MgCl2, 24 mM NaHCO3 and 1 g/L of BSA (pH 7.4)]. Cells were then exposed to 2.8 mM and 22.2 mM glucose for 60 min. At the end of the incubation period, the supernatant was collected and insulin levels in the media were measured using rat/mouse insulin ELISA kit EZRMI-13 K (Millipore Corporation, Billerica, MA, USA). The results were normalized using violet crystal optical density. Crystal violet is a triarylmethane dye that binds to DNA in nuclei. During the assay, dead detached cells are washed away. The remaining attached live cells were then stained with Crystal violet. After a wash step, the Crystal violet dye is solubilized and measured by absorbance at 595 nm (Feoktistova, Geserick, & Leverkus, 2016).

Evaluation of the mitochondrial respiration rates

The oxygen consumption rate (OCR), as an indicator of mitochondrial functionality, was determined using an XF-24 Extracellular Flux Analyzer (Seahorse Bioscience, Billerica, MA, USA). After the 48 h incubation period with the extracts and 1 h before oxygen consumption measurements, the cell media were replaced by the assay medium (complete RPMI with no phenol red, 2 mM glucose) for 60 min at 37 °C (on CO_2) before loading into a Seahorse XFp Extracellular Flux Analyzer. During these 60 min, cartridge ports containing the oxygen probes were loaded with the different compounds injected during the assay to measure respiration under several conditions. Basal respiration was recorded for 15 min, at 5 min intervals, until system stabilization. Carbonyl cyanide 4-trifluoromethoxyphenylhydrazone (FCCP, mitochondrial uncoupler) and Oligomycin (ATP synthase inhibitor) were used at final concentrations of 1 μM and Antimycin A plus Rotenone (mitochondrial respiration inhibitors) at 0.5 μM. All respiratory modulators were used at ideal concentrations titrated during preliminary experiments (data not shown) according to manufacturer's recommendations. The complete analyses of OCR by Seahorse thus allowed us to measure OCR in the following conditions: 1) Basal respiration: represents the oxygen consumption used to meet cellular ATP demand plus respiration to re-establish membrane potential dissipation due to mitochondrial proton leak. This is calculated as [$OCR_{no\ additions} - OCR_{AA/Rot}$]; 2) ATP Production: represents the decrease in oxygen consumption rate upon injection of the ATP synthase inhibitor oligomycin [$OCR_{Basal} - OCR_{Oligo}$]. Thus, this measure represents the portion of basal respiration that is being used to drive ATP production; 3) H+ (Proton) leak: Part of the basal respiration that is not coupled to ATP production. Several factors may alter proton leak, including the lipid and protein composition and activity of mitochondrial inner membrane. This measure is calculated as [$OCR_{oligo} - OCR_{AA/Rot}$]; 4) Maximal respiration: represents the maximal oxygen consumption rate attained by adding the uncoupler FCCP. FCCP dissipates the proton gradient and, thus, forces the respiratory chain to operate at maximum capacity to re-establish the physiological mitochondrial membrane potential. This measure is calculated as [$OCR_{FCCP} - OCR_{AA/Rot}$]; 5) Spare respiratory capacity: This measurement indicates the capability of the cell to respond to an energy demand as well as how closely the cell is to the theoretical maximum respiration. It is calculated as [$OCR_{FCCP} - OCR_{Basal}$]; 6) Non-mitochondrial respiration: OCR that persists due to a subset of cellular enzymes that consume oxygen after the addition of the respiration inhibitors Rotenone + Antimycin A. This is important to get an accurate measure of mitochondrial respiration.

Superoxide anion radical ($SO_2^{\bullet-}$) production

INS-1E cells were seeded in a 96-wells plate with μCLEAR® bottom (Greiner Bio-one, 655090) at the density of 10,000 cells/well and incubated at 37 °C, 5% (v/v) CO_2. After the 48 h incubation period with the extracts, medium was replaced, and cells were washed with PBS. Then, cells were incubated with 2 μM dihydroethidium (DHE, Thermo Fisher Scientific, D1168) or 10 μM MitoSOX™ Red Mitochondrial Superoxide Indicator (Thermo Fisher Scientific, M36008), both diluted in PBS solution, at 37 °C, 5% (v/v) CO_2 during 10 min. Next, cells were incubated with 5 μg/mL Hoechst 33342 (Thermo Fisher Scientific, H3570) in PBS solution during 15 min. Cells were kept in PBS solution and imaged in the ImageXpress Micro Confocal High Content Imaging System (Molecular Devices) using the TexasRed and DAPI fluorescence filters.

2.4.4. Statistical analyses

Data are expressed as means ± S.E.M of three independent experiments, with 3–4 technical replicates of each group in each experiment. Statistical analyses were performed using one-way ANOVA with Bonferroni correction for multiple testing.

3. Results and discussion

Several studies demonstrated that chronic exposure of β-cells to high glucose levels results in β-cells dysfunction and death (Armstrong et al., 2018; Kaiser, Leibowitz, & Nesher, 2003; Kim et al., 2005). In addition, during progression of type 2 diabetes, glucotoxicity contributes to advancing β-cells failure and development of overt diabetes (Maedler, Oberholzer, Bucher, Spinas, & Donath, 2003). In this scenario, we evaluated the ability of two *Solanum* extracts to protect against this high glucose induced β-cells dysfunctions. For this purpose, we employed the INS-1E cell line as an experimental model. According to Merglen et al. (2004), this cell line is considered a valuable beta-cell model because of the ability of these cells to dose-dependently secrete insulin when stimulated with increasing concentrations of glucose.

Both fruits are quite different concerning their physical–chemical properties as reported in our previous work (Pereira et al., 2019). Herein, it is also the first time that the alkaloid composition of juá-açu (*Solanum oocarpum* Sendtn) was investigated. Molecular network of fruit crude extracts (Fig. 1, C) reveals that juá-açu ripest pulp crude extract (JAC) was enriched in kukoamines (cluster A), while fruta-do-lobo crude extract (FLC) was enriched in glycoalkaloids (cluster B). Also, the major compounds of both extracts were tentatively identified, as shown in Table S1.

Both fruits have different alkaloids composition; therefore, we used both for cell toxicity assays. However, since the crude extract contains other compounds such as phenolics, flavonoids, lipids, and sugars, we performed a semi-purification focusing on enriching the alkaloid classes.

3.1. Alkaloids fractionation and cell death evaluation

We used different approaches for each fruit fractionation, once kukoamines are very sensitive to pH and are degraded under an alkaline condition, we performed their enrichment via adsorption onto polyamide resin under neutral conditions. The adsorption occurs most likely due to the inter-attraction between their amino groups with the carbonyl groups of polyamides. Then, with a pH change for acidic condition, the desorption occurs (Cheung et al., 2015). For glycoalkaloids, we performed a solid–liquid extraction under acidic condition to favor alkaloid water-solubilization, followed by a liquid–liquid extraction in basic condition to enhance alkaloid extraction by the organic solvent and keep the phenolic compounds (acidic) in water. After fractionation, we were able to obtain a cleaner extract for both fruits (reducing content of sugars and phenolic compounds), as shown in Table S1. Regarding JAE, we recovered kukoamine derivatives and also unknown compounds identified as peptides derivatives (Li et al., 2015). These peptides were also present in the alkaloid fraction of *Lycopodium* (Tian, Hu, & Guo, 2016).

Before testing for mitochondrial function, ROS production, and insulin secretion, we evaluated possible toxicity by determining cell death of INS-1E cells treated with both enriched extracts. Thus, increasing concentrations of JAE and FLE were used to treat the cells for 24 h (Table 1). The penetration of the propidium iodide (PI) dye in dead INS-1E cells after treatments was observed under fluorescence microscopy. As observed in Table 1, cell death induction (% of PI positive cells) was severe after treatment with FLE, from 25 μM and higher concentrations, 88 % or more of the treated cells died. In addition, Hoechst 33258

Table 1
Quantification of INS1E cell death by Hoechst (HO, nuclei) and propidium iodide (PI, dead cells) dyes staining after treatments with juá-açu enriched extract (JAE, 1–500 μM) or fruta-do-lobo enriched extract (FLE, 0.5–100 uM).

JAE		FLE	
Concentration μM	**% cell death**	**Concentration μM**	**% cell death**
0.0	3.9 ± 1.9	0.0	5.2 ± 1.5
1.0	3.0 ± 2.0	0.5	2.5 ± 0.2
2.5	6.0 ± 1.0	1.0	3.7 ± 0.8
5.0	5.1 ± 0.9	2.5	5.0 ± 1.4
10.0	6.0 ± 1.0	5.0	6.0 ± 1.9
25.0	6.2 ± 0.9	10.0	8.0 ± 5.5
100.0	6.5 ± 0.9	25.0	95.0 ± 13.3
250.0	6.1 ± 0.7	50.0	105.0 ± 1.4
500.0	18.0 ± 4.0	100.0	105.0 ± 16.3

Zero dose (control) contains only vehicle (DMSO). Results are expressed as average of three independent assays, using 3–4 replicates of each condition in each assay.

staining showed that there were significant morphological changes in the nuclear chromatin after treatment with FLE, and it was not possible to continue the tests with this extract. Human toxicological studies have shown that solamargine and solasonine used at certain levels cause toxic effects such as cell-membrane disruption and acetylcholinesterase inhibition. In contrast, when the cells were treated with JAE, low cell death (~6 %) was observed up to 250 μM dose (Table 1). Even at the highest dose evaluated, 500 μM, less than 20 % of cell death was observed. Therefore, for the biological assays, the JAE 100 μM dose was chosen for biological activity tests.

3.2. Evaluation of in vitro biological activities of JAE

Due to their primordial function in aerobic metabolism, mitochondria are of central interest in the pathophysiology of diabetes. It is established that excessive metabolism of glucose generates reactive oxygen species (ROS), exceeding the antioxidant defense mechanisms of mitochondria. The oversupply of ROS may act at many different levels in the pancreatic β-cells leading to death. The electron transport chain in

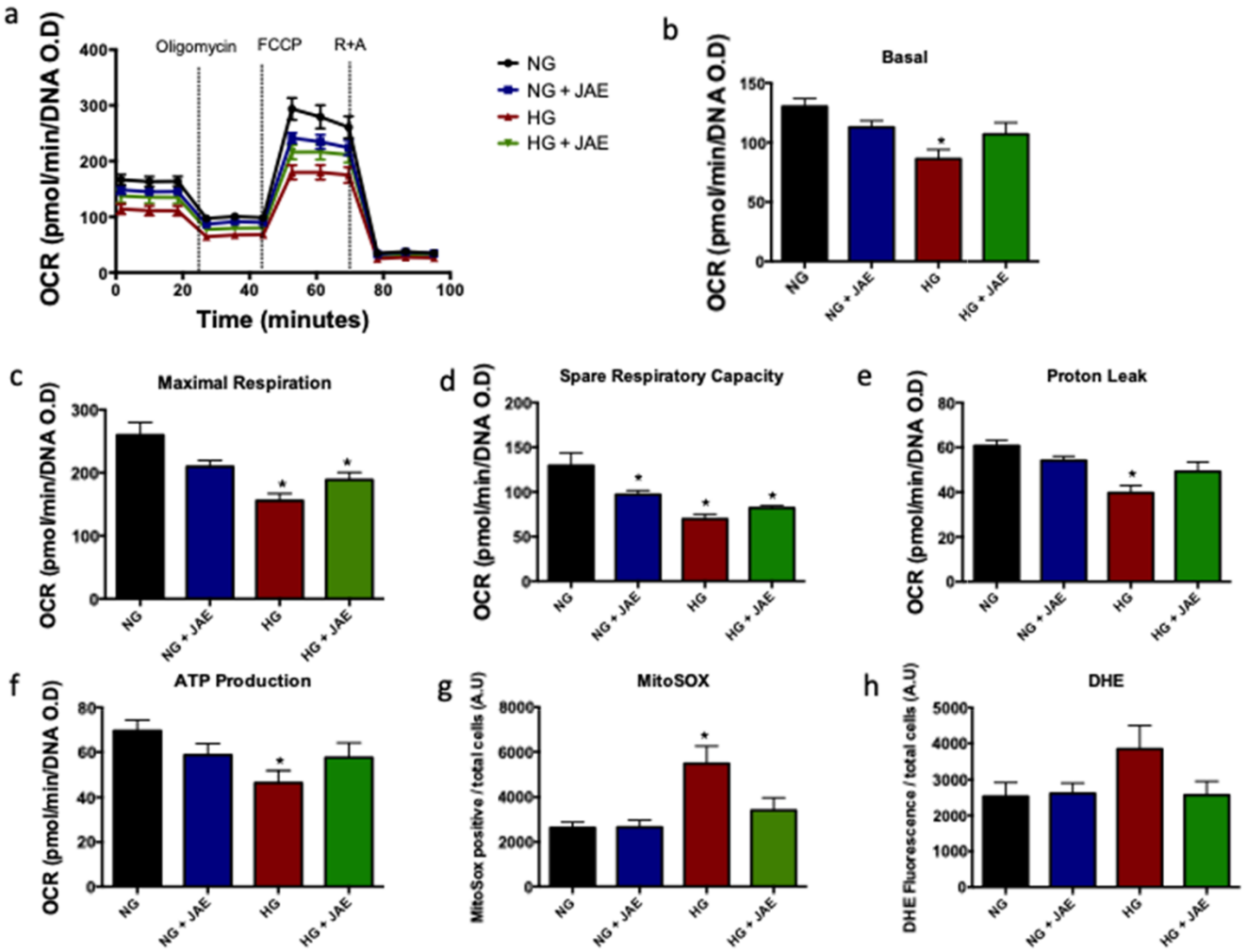

Fig. 2. JAE effects on INS-1E cells mitochondrial respiration (a-e) and cells superoxide anion radical ($SO_2^{\bullet-}$) production (f-g) after exposure to chronic high glucose conditions: INS-1E cells exposed to (NG) Normal glucose levels (11 mM), (HG) High glucose levels (30 mM), (NG + JAE) Normal (11 mM) plus JAE (100 uM), (HG + JAE) High glucose levels (30 mM) plus JAE (100 uM) for 48 h. (a) Representative traces of oxygen consumption rates (OCRs), Oligomycin, FCCP, rotenone/antimycin A (R + A) were sequentially injected in order to access mitochondrial respiratory in different conditions. Quantification of: (b) basal respiration, and respiration associated with (c) maximal respiration (d) spare respiratory capacity (e) proton leak and (f) ATP production. OCR values were normalized by the respectively DNA amount in each well. Three independent experiments in two well technical replicates per group were performed. (g) Mitocondrial derived and (h) total cell superoxide anion generation accessed by MitoSOX™ and dihydroethidium (DHE) reagents, respectively. Three independent experiments, with 3–4 technical replicates each, were analyzed by fluorescence microscopy. Statistical analyses were performed using one-way ANOVA with Bonferroni correction for multiple testing. Data are expressed as means ± S.E.M. ns, non-significant. * $p < 0.05$.

mitochondria is considered to be the most important intracellular source of ROS (Forbes-Hernández et al., 2014), thus, it is very important to ensure the integrity of the mitochondrial function as a strategy to avoid the overproduction of intracellular ROS and their consequent cellular damage. Based on this fact, the effects of JAE on mitochondrial functionality were investigated. After measuring basal respiration (oxygen consumption rates, OCR), cells were exposed sequentially to respiratory complexes activity modulators (oligomycin, FCCP and antimycin/rotenone), and OCR was monitored. In INS-1E cells chronically exposed to 30 mM glucose, basal OCR was markedly impaired ($p < 0.05$) compared with control cells maintained with 11 mM glucose, while treatment with JAE was able to protect mitochondria main bioenergetic function preventing this respiration reduction (Fig. 2 b). JAE was also able to protect mitochondrial functionality against glucotoxicity preserving not only basal respiration, but also respiration associated with proton leak and ATP production (Fig. 2 e-f). In addition, high glucose exposure increased the mitochondrial (Mitosox) and whole cell (DHE) superoxide anion production and JAE treatment was able to counteract these effects completely (Fig. 2 g-h). These data reinforce the beneficial effects of JAE against oxidative stress and inhibition of mitochondrial respiration evoked by glucotoxicity.

Insulin release is a complex and highly controlled process which is dependent on the stimulus-secretion coupling, whereby glucose catabolism within pancreatic β-cells generates the primary signal for secretion (Henquin, 2000). ATP generated mainly by mitochondria provides the key signal for closure of ATP-sensitive K^+ channels, subsequently causing membrane depolarization and activation of voltage-dependent Ca^{2+} channels (VDCCs), Ca^{2+} influx, and exocytosis of insulin vesicles (Ashcroft et al., 1994). Thus, mitochondrial functionality plays a critical role in stimulus-secretion coupling (Wiederkehr & Wollheim, 2012). In addition, excess of mitochondrial ROS formation may be harmful for mitochondria themselves, for insulin secretion and cell survival (Lorza-Gil et al., 2019). In this study, the insulin secretion was impaired, as expected, by chronic high glucose exposure (Fig. 3, NG vs HG). JAE treatment was able to restore insulin secretion levels (Fig. 3, HG vs HG + JAE).

In the last years, growing interest was devoted to studying the effects of natural compounds on mitochondrial functions. Li et al. (2019) investigated kukoamine B (KB), a major alkaloid from *Lycii Cortex*, which also shows antioxidant and anti-inflammatory properties. KB was able to lower glucose levels and inflammatory cytokines markers in animal studies without body weight-gain side effects (Li et al., 2019). Here we showed that JAE, rich in kukoamine alkaloids, is also able to protect mitochondrial function and glucose-induced insulin secretion against damage induced by high glucose exposure.

According to the results presented here, JAE could be proposed as a putative natural source of bioactive compounds protecting β-cells against oxidative damage induced by high-glucose levels, improving mitochondrial functionality and insulin secretion. However, some limitations of the present study must be acknowledged. A better purification and further analysis of JAE are still needed to evaluate which purified compounds are responsible for the observed effects. In addition, tests in more physiological models must be performed, such as primary β cell, pancreatic islets, and in vivo animal models. Once compound and best model are found, other mechanisms of action must be investigated and clarified. Nonetheless, these results are very exciting, and JAE rises as a potential source of compounds that could be used in prevention or as adjuvant therapy for diabetes.

4. Conclusion

Juá-açu alkaloid constituents were characterized for the first time and demonstrated a prolific production of kukoamine analogues. Additionally, two purification processes to enrich alkaloids classes (kukoamines and glycoalkaloids) were successfully employed to obtain distinct alkaloids composition from both fruits. FLE, rich in glycoalkaloids, showed a high toxic effect in cell death assays. However, JAE was able to protect beta-cells against oxidative stress, mitochondrial malfunction, and defective insulin secretion induced by high-glucose levels. These exciting results raise the possibility of using JAE as a source of bioactive compounds for prevention or adjuvant diabetes therapy.

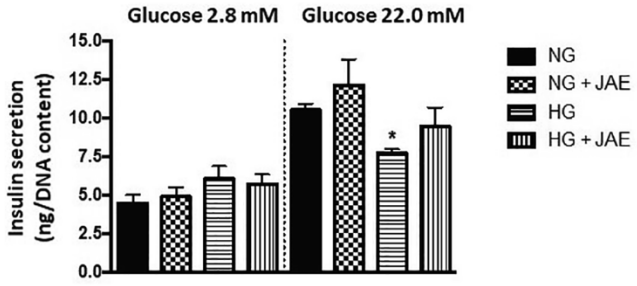

Fig. 3. JAE effects on INS-1E insulin secretion after exposure to chronic hyperglycemia conditions. INS-1E cells exposed to normal (NG) Normal glucose levels (11 mM), (HG) High glucose levels (30 mM), (NG + JAE) Normal (11 mM) plus JAE (100 uM), (HG + JAE) High glucose levels (30 mM) plus JAE (100 uM) for 48 h three technical replicates per group. Statistical analyses were performed using one-way ANOVA with Bonferroni correction for multiple testing. Data are expressed as means ± S.E.M. ns, non-significant. * $p < 0.05$.

CRediT authorship contribution statement

Ana Paula Aparecida Pereira: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Celio Fernando Figueiredo Angolini:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization, Supervision. **Jane Cristina de Souza-Sporkens:** Investigation, Methodology, Formal analysis, Writing - review & editing. **Tomaz Antonio da Silva:** Formal analysis, Investigation. **Helena Coutinho Franco de Oliveira:** Investigation, Writing - review & editing, Resources. **Glaucia Maria Pastore:** Conceptualization, Resources, Supervision, Funding acquisition, Writing - review & editing.

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Compliance with Ethical Standards.

Conflict of interest: The authors declares that he has no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: Publication.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2021.110589.

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