Journal of Functional Foods xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Effect of dietary *Acrocomia aculeata* kernel oil rich in medium chain fatty acids on type 2 diabetic rats

Ângela Alves Nunes [a,f,*], Danieli Fernanda Buccini [f], Jeandre Augusto dos Santos Jaques [b], Luciane Candeloro Portugal [b], Rita Cássia Avellaneda Guimarães [c], Simone Palma Favaro [d], Ruy de Araújo Caldas [e], Cristiano Marcelo Espinola Carvalho [f]

[a] Postdoctoral Student in Program in Biotechnology, Universidade Católica Dom Bosco, Campo Grande, MS, Brazil
[b] Institute of Biosciences, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil
[c] Post-Graduate Program in Health and Development in the Mid-West Region, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil
[d] Empresa Brasileira de Pesquisa Agropecuária - Embrapa, PqEB, W3 Norte - Asa Norte, Brasília, DF, Brazil
[e] Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil
[f] Post-Graduate Program in Biotechnology, Universidade Católica Dom Bosco, Campo Grande, MS, Brazil

ARTICLE INFO

Keywords:
Macauba
Lauric acid
Cholesterol
Insulin resistance
Diabetes
Hyperglycemia

ABSTRACT

This study evaluated the effects of dietaries formulated with kernel oil of *Acrocomia aculeata* (AKO), rich in medium chain fatty acids (MCFA), as partial substitute of carbohydrate calories upon blood glucose, lipid profile, insulin secretion and insulin sensitivity in diabetic rats. Overall, the dietary with any AKO dose reduced hyperglycemia, ameliorated insulin secretion, lowered insulin resistance by model HOMA-IR and HOMA-β and augmented pancreatic beta cells functionality, restored the number of pancreatic β-cell in the diabetic rats and increased it in the non-diabetic rats. In addition, AKO fed rats showed reducing triglycerides, lower density lipoprotein-cholesterol and increasing high density lipoprotein-cholesterol levels, and improved hepatic function markers. Those findings suggest AKO was effective to ameliorate the health of diabetic rats.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease which arises from complex interactions between multiple genetic and environmental factors as well as due to one's lifestyle. T2DM is characterized by either high blood glucose level (hyperglycemia), disorders in the metabolism of carbohydrates, lipids and proteins due to defective insulin secretion, or action or by both (American Diabetes Association, 2020; International Diabetes Federation, 2019; Bene, Hadzsiev, & Melegh, 2018; Subramanian & Prasath, 2014).

The worldwide increase of diabetes is a public health issue. According to the International Diabetes Federation, there is an 8.3 percent global prevalence among adults (aged 20–79years), which translates to 463 million adults having diabetes in 2019. These numbers are predicted to increase 9.6 percent by 2045, reaching 700 million adults (International Diabetes Federation, 2019). Brazil has 16.8 million people affected by T2DM, which accounts for 8.0 percent of the total population. This ranks Brazil in fifth for the countries with the largest prevalence of case worldwide, and in first among South and Central American countries (International Diabetes Federation, 2019).

Most patients with T2DM cannot maintain long-term glycemic levels under control. Several approaches have been applied in order to stabilize these levels, such as suitable diet and physical exercise and the use of hypoglycemic drugs in more severe cases. However, such drugs can cause several side effects (Veerapur, Prabhakar, Kandadi, Srinivasan, & Unnikrishnan, 2010). Therefore, the demand for new antidiabetic drugs and treatment approaches is increasing, especially for nutraceuticals with milder side effects and higher antidiabetic potential.

Abbreviations: AKO, *Acrocomia aculeata* kernel oil; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMC, body mass index; BW, body weight; HDL-C, high density lipoprotein cholesterol; HFD, high fat diet; HOMA-IR, homeostasis model of assessment of insulin resistance; HOMA-β, homeostatic model assessment pancreatic beta cells functional capacity; LCFAs, long chain fatty acids; LDL-C, low density lipoprotein cholesterol; MCFAs, medium chain fatty acids; MCT, medium chain triglycerides; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triacylglycerol; VLDL-C, very low density lipoprotein cholesterol.

* Corresponding author at: Post-Graduate Program in Biotechnology Universidade Católica Dom Bosco, Zip Code 79117-010, Campo Grande, MS, Brazil.
E-mail address: nunysnutri@yahoo.com.br (Â.A. Nunes).

https://doi.org/10.1016/j.jff.2020.104295
Received 27 July 2020; Received in revised form 9 November 2020; Accepted 18 November 2020

Pharmacological intervention with natural product-based ingredients is claimed as a healthier alternative to treat diabetes and could have greater effectiveness and lower cost. Products from the wild plant biodiversity have shown some effectiveness to treat diabetes in traditional medicine (Ngala, Ampong, Sakyi, & Anto, 2016; Kochikuzhyil, Devi, & Fattepur, 2010; Naskar et al., 2011; Freitas de Lima et al., 2017; Baldissera et al., 2017; da Silva et al., 2019). In our research group, we have been looking at alternative sources other than carbohydrates to supply energy as a strategy to mitigate the deleterious effects of T2DM.

A promising source are the vegetable oils rich in medium chain fatty acids (MCFAs), meaning the fatty acids whose carboxylic chain is within 6–12 carbons. Those fatty acids have been shown to improve factors associated with type 2 diabetes by lowering total cholesterol and density lipoprotein (LDL) levels, lowering aspartate transaminase (AST) activity, and lowering body weight (BW) and body mass index (BMC) (Airhart et al., 2016). The role MCFAs play is likely connected to their specific mechanisms of absorption, transport, and mitochondrial metabolism, which diverges from long chain fatty acids. MCFAs have an entrance to the mitochondrial matrix that bypasses the chylomicrons lymphatic route and is independently the L-Carnitine mediated transport, making it a unique fatty acid. Additionally, the transport is not under L-malonyl-CoA metabolic control and the deposit in the adipose tissues is low (Bach & Babayan, 1982; Bremer, 1983; Marten, Pfeuffer, & Schrezenmeir, 2006; Schönfeld & Wojtczak, 2016).

The metabolic pathways of MCFAs could explain their effects upon reduced adiposity and preserved insulin action in muscle and adipose tissue. This was observed in Wistar rats fed with a high fat diet rich in MCFAs 59% for 5 weeks, suggesting that this could prevent obesity and peripheral insulin resistance (Turner et al., 2009).

Other reports on humans and rodents have shown the benefits of diets supplemented with a high proportion of MCFAs instead of LCFAs. Some benefits include more efficient energy expenditure, higher fatty acid oxidation, lower adiposity, reducing fasting insulin level and amelioration of insulin resistance (HOMA-IR) (St-Onge, Bourque, Jones, Ross, & Parsons, 2003; St-Onge, Ross, Parsons, & Jones, 2003; Shinohara, Ogawa, Kasai, & Aoyama, 2005; Han et al., 2007; St-Onge & Bosarge, 2008; Montgomery et al., 2013; Airhart et al., 2016; Sung, Liao, & Chien, 2018).

Aiming to diversify the traditional vegetable oils portfolio, which are facing challenges due to environmental sustainability issues, non-traditional crops have arisen as promising sources. Macauba (*Acrocomia ssp*), for instance, is a palm tree native to tropical America found mostly in the wetlands (Pantanal) and Savannah like area (Cerrados) of Brazil (Henderson, Galeano, & Bernal, 1997). Its fruit yields pulp (APO) and kernel oils (AKO), whose fatty acid compositions are very distinguishable (Del Río et al., 2016). Lipid content of the kernel is around 60% (dry matter basis) and its fatty acid composition encompasses 59.9–75.6% saturated fatty acids. Among them, MCFAs accounts for approximately 50% of the total composition, and lauric acid (C12:0) is the most abundant, accounting for 32.0–45.4% (Coimbra & Jorge, 2012; Lescano et al., 2015; Del Río et al., 2016; Nunes et al., 2018; Magalhães, Tavares, & Nunes, 2020).

Macauba palm is receiving great interest not only in Brazil, but also abroad due to its high oil yield and sustainable production systems (Lieb et al., 2019; Tilahun et al., 2019; Colombo, Berton, Diaz, & Ferrari, 2018; Barbosa-Evaristo et al., 2018; de Lima, Carvalho, Meerow, & Manfrin, 2018; Moreira et al., 2018; Acrocomia Research Platform, 2019). In addition to picking from natural groves, commercial plantations of macauba are spreading in Brazil and in the near future, its oils will be available at large scale (Inocas, 2020; Soleá, 2020).

Macauba kernel oil could be an alternative source or partial replacement of energy supply for individuals carrying T2DM syndrome. Our previous report with macauba oil showed that rats, induced to T2DM who had AKO added to the diet to replace partially the total calories from carbohydrates, presented: weight loss, lower MCFAs deposition in the epididymal adipose tissue, and reduced blood glucose level (Nunes et al., 2018). In the present study, the effect of AKO was further evaluated upon serum lipid profile, insulin secretion, and insulin sensitivity in diabetic rats induced by high fat diet and low dose streptozotocin.

2. Material and methods

2.1. Fruit harvest and oil extraction

Ripen macauba fruits were collected directly from the bunch of macauba palm trees growing in Mato Grosso do Sul State, Central-West region of Brazil (22°05′44.6″S 55°20′50.5″W). The hard-coriaceous endocarp that covers the kernel was mechanically broken to deliver the kernel. The kernel oil was extracted by expeller press (Ecirtec Brand, Model MPE-40) followed by centrifugation at 1300*g* for 10 min (Quimis Brand, Model Q222TM104). This crude macauba kernel oil, from now on called AKO, was stored at −20 °C in amber glass flask after N_2 bubbling. The AKO was characterized and used in the experimental diets.

2.2. Profile of fatty acids in the diets and Acrocomia aculeata kernel oil

The fatty acid methyl ester (FAME) composition was determined after converting the oil to fatty acid methyl esters as described by Nunes et al., 2018. Firstly, 2 mL of 7% boron fluoride (BF_3) methanolic solution, and 1 mL of toluene were added to the oil. The mixture was then heated to 100 °C for 45 min, cooled down to room temperature, followed by the addition of 5 mL of water, 3 mL of hexane, and 300 mg of sodium sulphate. The mixture was stirred and left to settle, after which the top layer was injected into a GC (Agilent 6890N, California) to obtain individual peaks of fatty acid methyl esters. The GC was equipped with a flame ionization detector (FID) and a polar capillary column (HP 88, 0.25 mm internal diameter, 100 m length, and 0.25 mm film in thickness). Injector temperature was 225 °C and detector temperature was 285 °C. The initial column temperature was kept at 160 °C for 3 min, then increased to 190 °C at 3 °C/min for 6 min, followed by an increase to 230 °C at 6 °C/min and for 12 min. Total run time was 37.67 min. Individual FAME peaks were identified comparing their relative retention times with individual FAME standard (Supelco C8-C22, 99% pure). For additional control, samples were spiked with methyl undecanoate as an internal standard. Readings were analyzed using the Agilent Technologies Chemstation A09.01 Software.

2.3. Experimental animals

Male albino Wistar rats (n = 50; weighing within 150–170 g) were purchased from the Animal Facility of the São Paulo University (USP), Ribeirão Preto, SP, Brazil. All procedures were performed in accordance with the Ethical Principles in Animal Research in the Guide for the Care and Use of Laboratory Animals (Concil, 2010). Animal maintenance followed the guidelines of the Committee on Animal Experiments of the Dom Bosco Catholic University whose Committee for Ethics in Animal Experimentation approved these experiments under protocol N° 003/2015. The rats were housed in standard sanitized polypropylene cages containing paddy husk as bedding (2 rats/cage) and maintained under controlled conditions, temperature of 22 ± 2 °C and 12/12 h light–dark cycle. The animals were acclimatized for a week by being fed with standard laboratory rat chow (Nuvilab® CR-1, Nuvital, PR, Brazil) and water ad libitum.

2.4. Induction of type 2 diabetes by high fat diet and low-dose streptozotocin

The rats were allocated into two dietary regimens. Diabetes was induced by feeding with high fat diet - HFD (Table 1) (58% calories from fat - lard, 25% calories from protein and 17% calories from carbohydrate

Table 1
Diet composition and profile of fatty acids.

Ingredients (g kg^{-1} of diet)*	High Fat Diet (HFD)	Standard Diet (AIN93M)	AKO Low dose (Diet 1)	AKO High dose (Diet 2)
Casein	335.0	140.0	140.0	140.0
Cornstarch	78.5	465.7	465.7	195.7
Dextrinized cornstarch	50.0	155.0	155.0	155.0
Sucrose	90.0	100.0	100.0	100.0
Soybean oil	40.0	40.0	0.0	0.0
Lard	306.0	0.0	0.0	0.0
Acrocomia aculeata kernel oil	0.0	0.0	40.0	160.0
Fiber	50.0	50.0	50.0	50.0
Mineral mix	35.0	35.0	35.0	35.0
Vitamin mix	10.0	10.0	10.0	10.0
L-Cystine	1.8	1.8	1.8	1.8
Choline bitartrate	2.5	2.5	2.5	2.5
Tert-butylhydroquinone (TBHQ)	0.048	0.008	0.008	0.048
Energy of total kcal (percent)				
Carbohydrate	17.0	75.9	75.9	47.9
Protein	25.1	14.7	14.7	14.7
Fat	57.9	9.4	9.4	37.4
Fatty acid composition (percent)				
Saturated				
Caprilic, C8:0	–	–	5.42 ± 0.07	6.46 ± 0.06
Capric, C10:0	–	–	4.16 ± 0.20	4.49 ± 0.07
Lauric, C12:0	–	–	40.35 ± 1.88	41.88 ± 1.60
Myristic, C14:0	1.31 ± 0.01	0.11 ± 0.00	9.07 ± 0.09	9.13 ± 0.29
Palmitic, C16:0	22.91 ± 0.09	11.05 ± 0.18	7.66 ± 0.22	7.17 ± 0.02
Heptadecanoic, C17:0	0.46 ± 0.00	0.10 ± 0.00	–	–
Stearic, C18:0	13.49 ± 0.11	4.41 ± 0.03	3.22 ± 0.18	2.96 ± 0.19
Arachidic, C20:0	0.26 ± 0.00	0.44 ± 0.01	0.19 ± 0.02	0.19 ± 0.03
∑ SFA	**38.43**	**16.11**	**70.07**	**72.28**
∑ MCFA	–	–	**49.93**	**52.83**
Monounsaturated (MUFA)				
Palmitoleic, C16:1	1.40 ± 0.02	–	–	–
Oleic, C18:1 (ω 9)	32.35 ± 0.06	24.43 ± 0.11	27.83 ± 1.32	26.75 ± 1.33
Cis-11-eicosenic, C20:1	0.64 ± 0.02	0.25 ± 0.01	0.16 ± 0.00	0.14 ± 0.02
∑ MUFA	**34.39**	**24.68**	**27.99**	**26.89**
Polyunsaturated (PUFA)				
Linoleic, C18:2 (ω 6)	20.03 ± 0.07	48.12 ± 0.09	4.22 ± 0.19	3.47 ± 0.16
Linolenic, C18:3 (ω 3)	1.39 ± 0.01	5.23 ± 0.00	–	–
∑ PUFA	**21.42**	**53.35**	**4.22**	**3.47**

* The diets were formulated in accordance to the American Institute of Nutrition (AIN93M) and were produced by the PragSolucões® Co. Company, Brazil. Standard diet AIN93M; High Fat Diet – HFD; AKO Low dose: 40 g of AKO kg^{-1} of diet 1; AKO High dose: 160 g of AKO kg^{-1} of diet 2. Abbreviations: AKO - *Acrocomia aculeata* kernel oil; SFA - saturated fatty acids; MCFA - medium chain fatty acids.

as the percentage of total kcal) (Srinivasan, Viswanad, Asrat, Kaul, & Ramarao, 2005). The nondiabetic rats were fed with a Standard diet (Table 1), as per the recommendations of the American Institute of Nutrition (AIN93M) (Reeves, Nielsen, & Fahey, 1993). Both groups were fed accordingly over three weeks. Afterwards, both groups were left fasting overnight (12 h). For induction of diabetes, in the HFD groups, a single low dose of streptozotocin (STZ) was administered intraperitoneally. The STZ dose (Sigma-Aldrich, St. Louis, MO, USA. Ref S0130) involved 35 mg/kg body weight dissolved in freshly prepared sodium citrate buffer (0.1 M, pH 4.5). The vehicle was administered in the nondiabetic animals. After 6 h of STZ injection, the rats were fed again with their respective diet. In order to prevent fatal hypoglycemia, water was replaced by a 5% dextrose solution (Sigma-Aldrich, St. Louis, MO, USA. Ref. D9434) for the following 24 h. At the end of the fourth week, blood glucose level was measured in previously fasted animals (12 h), following the enzymatic glucose oxidase method using a commercial glucometer (Accu-Chek®Active, Roche Diagnostic, Mannheim, Germany). Animals with blood glucose ≥250 mg/dL were considered diabetic and selected for the study. There was no death among the experimental groups along the assays.

2.5. Experimental design

The animals were randomly divided into five groups (eight rats each n = 8):

Group (NC): Normal control received (Standard diet AIN93M);
Group (DBC): Diabetic control received (Standard diet AIN93M);
Group (DAL): Diabetic rats + AKO low dose (40 g of AKO kg^{-1} of diet 1);
Group (DAH): Diabetic rats + AKO high dose (160 g of AKO kg^{-1} of diet 2);
Group (NAH): Non-diabetic rats + AKO high dose (160 g of AKO kg^{-1} of diet 2).

Diets were developed by following the American Institute of Nutrition (AIN93M) guides, exchanging the fat source, and providing partial replacement to the carbohydrate's energy source with AKO, as described in (Table 1).

Body weight, food, and water intake were measured every two days throughout 28 days using a semi-analytical balance. Fasting blood glucose levels were monitored on days 0, 7, 14, 21 and 28 using an Accu-Chek® Active glucometer (Roche Diagnostics, Mannheim, Germany). At the end of the treatments (28 days), the rats were left fasting (12 h), anesthetized with isoflurane for blood collection, and sacrificed by cervical dislocation. Blood was collected in tubes with or without anticoagulants (Vacuplast®collect line), and centrifuged at 1900*g* for 15 min. The plasma and serum were separated, stored at −20 °C, and used for biochemical analysis. The whole pancreas was excised and washed with cold saline, dried, and weighed. Part of the tissue was sliced and stored at −80 °C while the remainder was used in the histological examination.

2.6. Biochemical analysis

Fasting blood glucose was estimated in plasma. Total cholesterol (TC), triacylglycerol (TG), high density lipoprotein cholesterol (HDL-C), creatinine, urea, albumin, total protein, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were estimated in serum by diagnostic kit (In Vitro Diagnóstica Ltda, Itabira, MG, Brazil). Low density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated according to Friedewald, Levy, and Fredrickson (1972).

2.7. Enzyme-linked immunosorbent assay for insulin

Serum insulin levels were measured by enzyme-linked immunosorbent assay (ELISA) method using an ultrasensitive rat insulin ELISA kit (Crystal Chem. Inc., Downers Grove, IL 60515, USA) in a multi plate ELISA reader (Spectrophotometer, Thermo Fisher Scientific Oy, Vantaa – Filand). This assay had 100% cross-reactivity to rat insulin.

2.8. Homeostatic model assessment HOMA-IR and HOMA-β

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) and Homeostatic Model Assessment Pancreatic beta cells functional capacity (HOMA-β) were calculated according to the following formula by Matthews et al. (1985).

$$\mathbf{HOMA-IR} = \text{Fasting insulin level } (\mu U/mL) \times \text{Fasting blood glucose } (mg/dL)/405$$

$$\mathbf{HOMA}-\beta(\%) = 20 \times \text{Fastinginsulinlevel}(\mu U/mL)/\text{Fastingbloodglucose}(mmol/L) - 3.5$$

2.9. Histological examination of the pancreas

Pancreas samples were fixed in 10% buffered formalin, dehydrated, embedded in histological paraffin, sectioned (5 μm) in semi-serials cuts, and mounted on glass slides. The tissue sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). The morphometric analyses was performed at different magnifications in the non-serial pancreatic sections using hematoxylin and eosin (H&E) to estimate (a) the diameter of the pancreatic islets; (b) the number of islets in each section of the pancreas; (c) the number of β-cells of the pancreatic islets. The diameter of the pancreatic islets were measured for each animal (n = 5 animals/group) using 20 digital images (TIFF 8-bit color, 3264 × 2448 pixels) 200x magnification from each animal (n = 5 animals/group) obtained with a light microscopy (Primo Star ZEISS, Germany). Sections were analyzed at 100 μm intervals to avoid measuring the same islet twice. The program used to measure the diameter of the pancreatic islets was Image J (Image Processing and analysis in Java). Quantitative analyses of the pancreatic islets number were performed using digital images (Leica DM 500 Microscope) 50x magnification from 5 semi-serial sections of 5 μm from each animal (n = 5 animals/group). The β-cells were determined by direct counting method at 1000× magnification. Only islets containing 15 or more endocrine cells were measured. Stained sections were observed under the light microscope (Primo Star ZEISS, Germany) and by a Nikon D3100 camera coupled to the microscope. All analyses were performed in a double-blind test.

2.10. Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by the Tukey test as the post-test. The results were expressed as mean ± standard error of the mean (SEM), using software GraphPad Prism® version 5.00. $P < 0.05$ was statistically significant.

3. Results

3.1. Effect of Acrocomia aculeata kernel oil on fasting blood glucose, serum insulin and homeostatic model assessment HOMA-IR and HOMA-β

Fig. 1 shows the levels of fasting blood glucose (A), serum insulin (B), HOMA-IR (C) and HOMA-β (D) of the experimental animals. Induction of T2DM in the experimental rats was confirmed by presence of a high fasting blood glucose level. Moreover, the diabetic induced rats presented increasing plasma glucose (Fig. 1A) and lower serum insulin (Fig. 1B). The diabetic rats treated with AKO, whether DAL or DAH groups, showed a significant decrease in the fasting glucose level, 49.5% and 47.1% respectively (Fig. 1A). The serum insulin levels and β-cell function (HOMA-β) of the DBC group were significantly ($p < 0.05$) lower compared to the NC group. Rats treated with AKO in both DAL and DAH groups recorded significantly ($p < 0.05$) higher insulin levels and β-cell function than the DBC group (Fig. 1B, 1D). Indeed, the NAH group had a significantly ($p < 0.05$) higher insulin level than normal rats (NC) group.

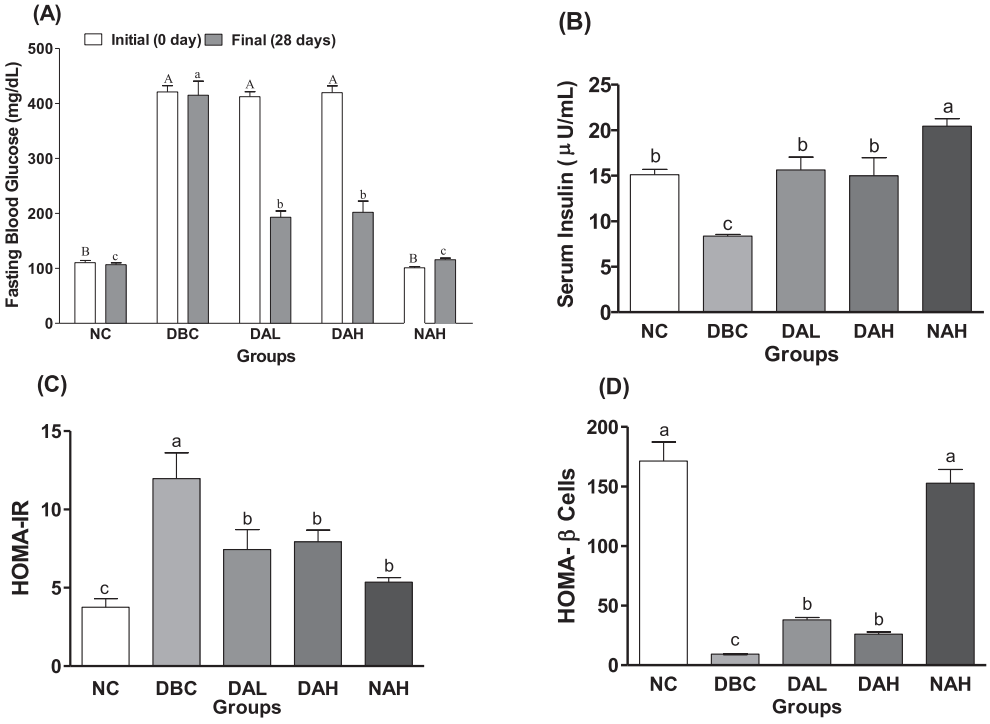

Fig. 1. Effect of *Acrocomia aculeata* kernel oil on fasting blood glucose (A), serum insulin (B), HOMA-IR (C) and HOMA-β (D). Data are expressed as mean ± SEM (n = 8). Means with different superscripts within the row are significantly different ($p < 0.05$). One-way ANOVA followed by Tukey post-hoc test. NC: normal control diet (Standard diet AIN93M); DBC: diabetic control diet (Standard diet AIN93M); DAL: Diabetic rats + low dose – 40 g of AKO kg^{-1} of diet 1; DAH: Diabetic rats + high dose – 160 g of AKO kg^{-1} of diet 2; NAH: Non-diabetic rats + high dose – 160 g of AKO kg^{-1} of diet 2.

HOMA-IR of diabetic rats showed a significant elevation ($p < 0.05$), but the insulin resistance in both DAL and DAH groups improved (Fig. 1C).

3.2. Effect of AKO on fasting lipids

The levels of lipid profiles in the control and experimental groups were shown in Table 2. In this study, the levels of TC, TG, LDL-C and VLDL-C in the DBC group were significantly higher ($p < 0.05$) and the level of HDL-C was significantly lower ($p < 0.05$) in comparison to NC group. On the other hand, treatment with AKO for 4 weeks in diabetic rats restored lipid serum profile compared to the DBC group. The TC level was the only one not restored.

3.3. Hepatic and renal function markers

Table 3 shows significant increase ($p < 0.05$) in the activities of serum AST and ALT of the DBC group compared to NC group. At the end of the experimental period, a significant decrease of the AST and ALT serum levels in the DAL and DAH groups, compared to DBC group, was observed. No significant differences ($p > 0.05$) in the serum levels of creatinine and total protein of all experimental groups were seen. However, urea levels increased significantly ($p < 0.05$) in all diabetic groups (DBC, DAL and DAH) compared to the NC and NAH groups. In addition, albumin levels were lower in the DAH and NAH groups at the end of the experimental period.

3.4. Morphometric pancreatic analysis

The results of the morphometric analysis revealed a significant reduction in the islet area and number in all T2DM rats compared to NC group (Fig. 2 A, B). The number of β-cells/islet was reduced in the DBC group, however in the DAL and DAH groups showed a significant increase ($p < 0.05$) (Fig. 2C).

4. Discussion

In this study, T2DM was induced in Wistar rats fed with a high fat diet (HFD) for three weeks followed by a low dose of streptozotocin (STZ) (Nunes et al., 2018). HFD leads to insulin resistance in peripheral tissues due to fat deposit, known as lipotoxicity. Low STZ dose partially damages pancreatic beta cells leading to the breakage of DNA strands. Cell death is related to alkylation of DNA and, consequently, an increase in blood sugar level (Elsner, Guldbakke, Tiedge, Munday, & Lenzen, 2000). Both HFD and STZ mimic the human syndrome T2DM (Reed et al., 2000; Srinivasan, Viswanad, Asrat, Kaul, & Ramarao, 2005).

Table 2
Effect of *Acrocomia aculeata* kernel oil on serum lipid profile.

Parameters	Experimental Groups[1] NC	DBC	DAL	DAH	NAH
Serum (mg/dL)					
Total cholesterol	71.21 ± 2.35^{b}	100.5 ± 4.80^{a}	92.79 ± 2.27^{a}	96.82 ± 6.07^{a}	81.64 ± 2.74^{b}
Triacylglycerol	83.03 ± 2.72^{b}	181.91 ± 3.85^{a}	88.54 ± 3.76^{b}	89.31 ± 8.64^{b}	82.94 ± 5.54^{b}
HDL-C	45.60 ± 4.01^{a}	21.82 ± 1.30^{b}	47.74 ± 3.32^{a}	48.21 ± 5.00^{a}	47.67 ± 2.37^{a}
LDL-C	11.28 ± 1.69^{c}	42.00 ± 0.82^{a}	27.35 ± 2.85^{b}	30.75 ± 1.85^{b}	17.38 ± 2.53^{c}
VLDL-C	16.61 ± 0.54^{b}	36.38 ± 0.77^{a}	17.70 ± 0.75^{b}	17.86 ± 1.73^{b}	16.59 ± 1.11^{b}

Data are expressed as mean ± SEM (n = 8). Means with different superscripts within the row are significantly different ($p < 0.05$). One-way ANOVA followed by Tukey post-hoc test. NC: normal control diet (Standard diet AIN93M); DBC: diabetic control diet (Standard diet AIN93M); DAL: Diabetic rats + low dose − 40 g of AKO kg^{-1} of diet 11; DAH: Diabetic rats + high dose − 160 g of AKO kg^{-1} of diet 2; NAH: Non-diabetic rats + high dose − 160 g of AKO kg^{-1} of diet 22.

Table 3
Effect of *Acrocomia aculeata* kernel oil on serum biochemical parameters.

Parameters	Experimental groups[1] NC	DBC	DAL	DAH	NAH
AST (U/L)	81.85 ± 8.57^{b}	132.9 ± 1.22^{a}	72.31 ± 6.60^{b}	80.77 ± 7.25^{b}	72.24 ± 7.32^{b}
ALT (U/L)	56.97 ± 3.26^{b}	82.24 ± 6.32^{a}	48.07 ± 6.24^{b}	57.33 ± 6.07^{b}	26.69 ± 1.58^{c}
Creatinine (mg/dL)	0.45 ± 0.02^{a}	0.51 ± 0.01^{a}	0.41 ± 0.01^{a}	0.43 ± 0.02^{a}	0.44 ± 0.04^{a}
Urea (mg/dL)	41.25 ± 3.28^{b}	80.44 ± 2.50^{a}	84.31 ± 1.51^{a}	90.24 ± 4.54^{a}	33.90 ± 1.55^{b}
Total Protein (g/dL)	5.57 ± 0.16^{a}	5.64 ± 0.27^{a}	6.13 ± 0.28^{a}	5.59 ± 0.13^{a}	6.25 ± 0.13^{a}
Albumin (g/dL)	3.97 ± 0.12^{a}	4.17 ± 0.13^{a}	4.20 ± 0.14^{a}	3.44 ± 0.10^{b}	3.65 ± 0.09^{b}

Data are expressed as mean ± SEM (n = 8). Means with different superscripts within the row are significantly different ($p < 0.05$). One-way ANOVA followed by Tukey post-hoc test. NC: normal control diet (Standard diet AIN93M); DBC: diabetic control diet (Standard diet AIN93M); DAL: Diabetic rats + low dose − 40 g of AKO kg^{-1} of diet 1; DAH: Diabetic rats + high dose − 160 g of AKO kg^{-1} of diet 2; NAH: Non-diabetic rats + high dose − 160 g of AKO kg^{-1} of diet 22.

The reduction of blood glucose level is the primary therapeutic goal for controlling diabetes. In the present study, the blood glucose level of diabetic rats was significantly decreased when treated with *Acrocomia aculeata* kernel oil (AKO), as shown by biochemical analysis (Fig. 1A). The maintenance of normal glycemia is paramount to reducing the risk of pathogenesis related to T2DM at the levels of macrovascular complications (coronary artery disease and peripheral arterial disease) and microvascular complications (chronic kidney disease – nephropathy, retinopathy and neuropathy) (Fowler, 2008; Rangel, Rodrigues, & De Sá, 2019).

The insulin sensitivity and β-cell function are estimated according to the HOMA model based on fasting plasma insulin and glucose concentration (Matthews et al., 1985). In our work, diets with added AKO were shown to increase the serum insulin, reduced insulin resistance, and augmented pancreatic β function, as the HOMA-IR and HOMA-β data show. Findings were corroborated by morphometric pancreatic data that showed a higher number of β-cells in the diabetic rats with AKO.

Ananda Prabu, Kumarappan, Christudas, and Kalaichelvan, (2012) evaluated the effect of bioactive phytochemical from *Biophytum sensitivum* aqueous solution for 28 days in T2DM Wistar rats and demonstrated the significant elevation of plasma insulin is probably related to the stimulation of insulin secretion from the existing pancreatic β cells. Therefore, the improvement of insulin sensitivity is an important therapeutic approach to treat type 2 diabetes.

The mass loss and functionality of pancreatic β-cells has been one of the major concerns in the pathogenesis of T2DM (Duru et al., 2020). Therefore, a therapeutic strategy to prevent the progression of T2DM is to maintain the functionality and the mass of β-cells (Aguayo-Mazzucato & Bonner-Weir, 2018).

In experimental animal model, Pujol et al., (2018) evaluated the beneficial effects of medium chain triglycerides (MCT) on β-cells and demonstrated that this could be a potential approach to treat and prevent T2DM because they are related to the improvement of glycemia in aged rats via higher insulin secretion.

The mechanisms encompassed in the beta-cell amelioration functionality, due to the higher MCFAs availability in the diet, could be related to three different metabolic processes. MCFAs are involved in both mitochondrial function improvement and the boost of the expression of genes linked to beta-cell function and insulin biogenesis (Pujol et al., 2018). In that report, MCFA-C10 promoted the expression of the gene GPR40, which leverages glucose insulin secretion in pancreatic beta-cells, and MCFA-C8 spurred mitochondrial ketogenesis. Those findings came out to the conclusion that MCFA offers a therapeutic

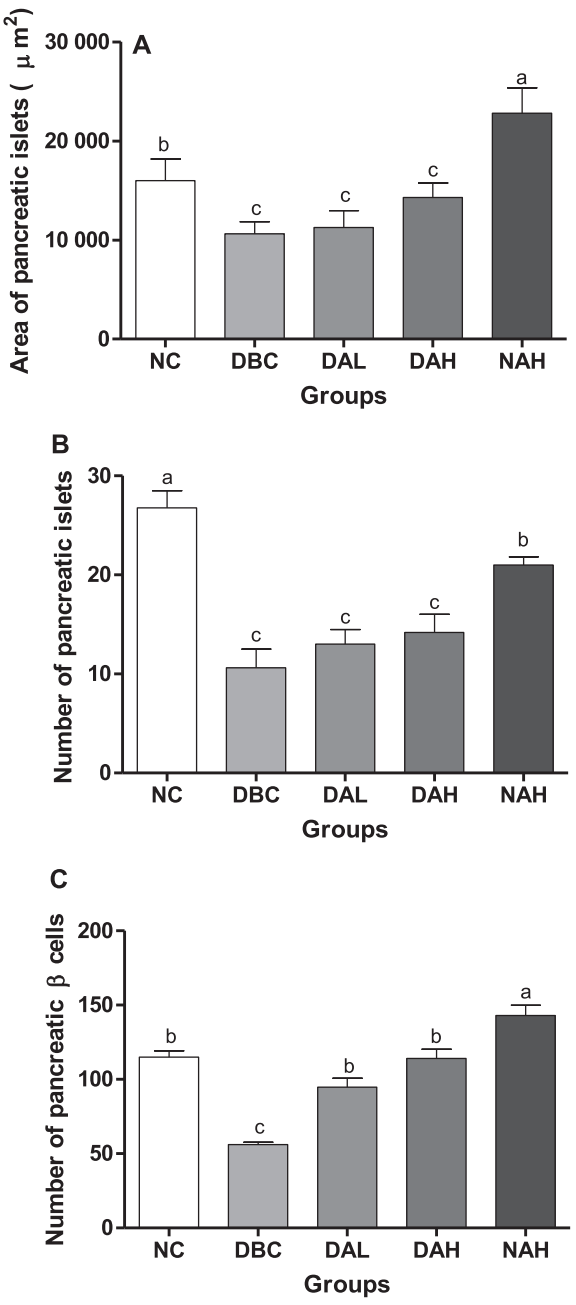

Fig. 2. Pancreatic morphometric analysis. Area of pancreatic islets (A), Number of pancreatic islets per pancreas area (B), Number of pancreatic β-cells (C). Data are expressed as mean ± SEM of values from 5 rats of each group. Means with different superscripts within the row are significantly different ($p < 0.05$). One-way ANOVA followed by Tukey post-hoc test. NC: normal control diet (Standard diet AIN93M); DBC: diabetic control diet (Standard diet AIN93M); DAL: Diabetic rats + low dose − 40 g of AKO kg^{-1} of diet 1; DAH: Diabetic rats + high dose − 160 g of AKO kg^{-1} of diet 2; NAH: Non-diabetic rats + high dose − 160 g of AKO kg^{-1} of diet 2.

advantage in the preservation of β-cell function as part of a preventative strategy against diabetes in at risk population. The third mechanism involves cell death due to the apoptosis process. MCFAs can play a protective role in beta cells because they do not induce the activity of the caspase-3 protease that is involved in the apoptosis process. While LCFAs showed a toxic effect on beta cells in parallel with increased caspase-3 activity (von Hanstein, Lenzen & Plötz, 2020).

Montgomery et al., (2013) demonstrated, in a study using mice model, that MCFA improves glucose tolerance, augments energy expenditure, and reduces adiposity in comparison to LCFA. In a study with humans carrying type 2 diabetic that consumed 18 g MCT per day for 90 days, reduced HOMA-IR, fasting insulin level and body weight when compared with the LCT group (Han et al., 2007).

Type 2 DM is also characterized by metabolic disorders including alterations in the levels of triglycerides, total cholesterol, and lipoproteins. In this study, it was also observed an increase in serum TG, TC, LDL-C and VLDL-C fractions along with a decreased HDL-C level in the diabetic control group. The HDL-C levels in diabetic and non-diabetic rats fed with 40 g (low dose) and 160 g (high dose) of AKO kg^{-1}, were found to be higher compared to the diabetic control group, an indication of a beneficial effect of the AKO. An increase in HDL-C usually results in decreased risk of coronary diseases (Park et al., 2019). While LDL-C levels were decreased in both DAL and DAH treatments showing a further beneficial effect of AKO.

Several studies have reported LDL-C as the most dangerous lipid and its oxidation increases its penetration in the arterial walls what results in the formation of atherosclerotic plaque lesions (Marinangeli, Varady, & Jones, 2006; Poznyak et al., 2020). In experiments with Sprague–Dawley rats, fed a diet containing virgin coconut oil (rich in lauric acid) for 45 days, showed an increase in HDL-C while LDL-C fraction decreased (Nevin & Rajamohan, 2004).

In addition, the serum total cholesterol in both diabetic control group and diabetic treatment with AKO were higher than the non-diabetic groups, indicating that AKO did not have any effect on the levels of total cholesterol. Similar results were obtained by Siddalingaswamy, Rayaorth, & Khanum, (2011), when diabetic rats were fed with cold virgin coconut oil.

Diet containing MCT oil reduced hepatic lipid synthesis by decreasing the activity of lipogenic enzymes and increasing hepatic lipolytic enzymes, consequently lowering serum TG (Schönfeld & Wojtczak, 2016). Higher levels of serum HDL-C in animals fed with MCT oil is related to the functionality of LDL receptors (Fernandez & West, 2005). LDL receptors are glycoproteins that recognize apoprotein B and it is controlled by glycosylation mechanism (Betteridge, 1989).

To evaluate possible hepatic dysfunction in T2DM rats serum activity of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured, high level of these markers indicate leakage from the hepatocytes into the bloodstream (Subramanian et al., 2015; Priscilla, Jayakumar, & Thirumurugan, 2015).

Table 3 shows significant increase in the activities of serum AST (62.4%) and ALT (44.3%) in the DBC group compared with NC group. Our results pointed out a considerable increase in both enzyme concentrations in rats induced to T2DM, this indicates liver damage, caused by STZ action, however diabetic rats fed with AKO showed reduced liver damage in both, low and high AKO dose.

In our previous report, it was demonstrated body weight loss in T2DM rats, this could be explained from two different point of view, the first could be due to lipolysis leading to reduction of adipose tissue, the second possibility might be related to proteolysis diminishing muscle tissue (Nunes et al., 2018).

In this respect, both creatinine and total protein serum concentrations were not altered, whereas urea levels increased significatively in all diabetic groups, showing higher proteolysis compared to the non-diabetic rats leading to ammonium production during the deamination steps which is immobilized into urea. Whereas a drop in concentration of serum albumin in both groups DAH and NAH was observed. This finding probably could be interpreted based on the assumption that determination of serum albumin using the bromocresol green method, in a condition of high levels of circulating fatty acids, underestimates albumin concentration.

As far as we know this is the first scientific evidence of AKO as hypoglycemic agent, protecting beta cells, improving their function ability, and acting as hepatoprotective. From our point of view this research opens a new avenue to pursue functional food for improving life condition and health of the population that utilizes the food potential of

biodiversity in general, mainly subjects carrying diabetic type 2.

Considering the establishment of the macauba value chain, these findings are very worthy, as kernel oil may be directed to a niche market for products with high added value, which is eager for products that are proven efficient and produced in a sustainable manner.

In summary, our data demonstrated that AKO fed to T2DM rats exerted hypoglycemic action, stimulated insulin secretion, improved pancreatic β cell function (HOMA-β), and decreased insulin resistance (HOMA-IR) it also had a positive effect on pancreatic islet morphology and on the number of β-cells. In addition, AKO decreased LDL-C and augmented HDL-C levels, which could reduce the risk of chronic diseases related to T2DM syndrome. Furthermore, AKO also had a hepatic protector effect lowering the levels of serum AST and ALT.

5. Ethics statement

All procedures were performed in accordance with the Ethical Principles in Animal Research in the Guide for the Care and Use of Laboratory Animals (Concil, 2010). Animal maintenance followed the guidelines of the Committee on Animal Experiments of the Dom Bosco Catholic University whose Committee for Ethics in Animal Experimentation approved these experiments under protocol N° 003/2015.

CRediT authorship contribution statement

Ângela Alves Nunes: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Danieli Fernanda Buccini:** Investigation, Visualization. **Jeandre Augusto dos Santos Jaques:** Resources, Visualization. **Luciane Candeloro Portugal:** Investigation, Visualization. **Rita Cássia Avellaneda Guimarães:** Resources, Visualization. **Simone Palma Favaro:** Resources, Writing - review & editing. **Ruy de Araújo Caldas:** Conceptualization, Resources, Writing - review & editing. **Cristiano Marcelo Espinola Carvalho:** Conceptualization, Resources, Writing - review & editing, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT).

References

Acrocomia Research Platform. (2019). Hohenheim Research Center for Global Food Security and Ecosystems (GFE). https://gfe.uni-hohenheim.de/en/acrocomia/ Access Apr 2020.

Aguayo-Mazzucato, C., & Bonner-Weir, S. (2018). Pancreatic β cell regeneration as a possible therapy for diabetes. *Cell Metabolism, 27*(1), 57–67. https://doi.org/10.1016/j.cmet.2017.08.007.

Airhart, S., Cade, W. T., Jiang, H., Coggan, A. R., Racette, S. B., Korenblat, K., … Peterson, L. R. (2016). A diet rich in medium-chain fatty acids improves systolic function and alters the lipidomic profile in patients with type 2 diabetes: A pilot study. *Journal of Clinical Endocrinology and Metabolism, 101*(2), 504–512. https://doi.org/10.1210/jc.2015-3292.

American Diabetes Association. (2020). Standards of medical care in diabetes 2020 ADA. *American Diabetes Association, 42*(479), 960–1010. https://doi.org/10.1192/bjp.111.479.1009-a.

Ananda Prabu, K., Kumarappan, C. T., Christudas, S., & Kalaichelvan, V. K. (2012). Effect of Biophytum sensitivum on streptozotocin and nicotinamide-induced diabetic rats. *Asian Pacific Journal of Tropical Biomedicine, 2*(1), 31–35. https://doi.org/10.1016/S2221-1691(11)60185-8.

Bach, A. C., & Babayan, V. K. (1982). Medium-chain triglycerides: An update. *American Journal of Clinical Nutrition, 36*(5), 950–962. https://doi.org/10.1093/ajcn/36.5.950.

Baldissera, M. D., Souza, C. F., Grando, T. H., Cossetin, L. F., Sagrillo, M. R., Nascimento, K., … Monteiro, S. G. (2017). Antihyperglycemic, antioxidant activities of tucumã oil (*Astrocaryum vulgare*) in alloxan-induced diabetic mice, and identification of fatty acid profile by gas chromatograph: New natural source to treat hyperglycemia. *Chemico-Biological Interactions, 270*, 51–58. https://doi.org/10.1016/j.cbi.2017.04.001.

Barbosa-Evaristo, A., Fernández-Coppel, I. A., Corrêa-Guimarães, A., Martín-Gil, J., Duarte-Pimentel, L., Saraiva-Grossi, J. A., … Martín-Ramos, P. (2018). Simulation of macauba palm cultivation: An energy-balance and greenhouse gas emissions analysis. *Carbon Management, 9*(3), 243–254. https://doi.org/10.1080/17583004.2018.1463783.

Bene, J., Hadzsiev, K., & Melegh, B. (2018). Role of carnitine and its derivatives in the development and management of type 2 diabetes. *Nutrition and Diabetes, 8*(1), 1–10. https://doi.org/10.1038/s41387-018-0017-1.

Betteridge. (1989). Diabetes, lipoprotein metabolism and atherosclerosis. *British Medical Bulletin, 45*(1), 285–311. https://doi.org/10.1093/oxfordjournals.bmb.a072317.

Bremer, J. (1983). Carnitine. Metabolism and functions. *Physiological Reviews, 63*(4), 1420–1480. https://doi.org/10.1152/physrev.1983.63.4.1420.

Coimbra, M. C., & Jorge, N. (2012). Fatty acids and bioactive compounds of the pulps and kernels of Brazilian palm species, guariroba (*Syagrus olerace*s), jerivá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*). *Journal of the Science of Food and Agriculture, 92*(3), 679–684. https://doi.org/10.1002/jsfa.4630.

Colombo, C. A., Berton, L. H. C., Diaz, B. G., & Ferrari, R. A. (2018). Macauba: A promissing tropical palm for the production of vegetable oil. D108, 1–9 *OCL - Oilseeds and Fats, Crops and Lipids, 25*(1). https://doi.org/10.1051/ocl/2017038.

Concil. (2010). National Research Council. Guide for the care and use of laboratory animals: National Academies Press. In: 8th edition revised. National Academy of Sciences, Washington, DC.

da Silva, P. V. B., Ramiro, M. M., Iriguchi, E. K. K., Corrêa, W. A., Lowe, J., Cardoso, C. A. L., … Muzzi, R. M. (2019). Antidiabetic, cytotoxic and antioxidant activities of oil extracted from *Acrocomia aculeata* pulp. *Natural Product Research, 33*(16), 2413–2416. https://doi.org/10.1080/14786419.2018.1446006.

de Lima, N. E., Carvalho, A. A., Meerow, A. W., & Manfrin, M. H. (2018). A review of the palm genus *Acrocomia*: Neotropical green gold. *Organisms Diversity and Evolution, 18*(2), 151–161. https://doi.org/10.1007/s13127-018-0362-x.

Del Río, J. C., Evaristo, A. B., Marques, G., Martín-Ramos, P., Martín-Gil, J., & Gutiérrez, A. (2016). Chemical composition and thermal behavior of the pulp and kernel oils from macauba palm (*Acrocomia aculeata*) fruit. *Industrial Crops and Products, 84*, 294–304. https://doi.org/10.1016/j.indcrop.2016.02.018.

Duru, K. C., Mukhlynina, E. A., Moroz, G. A., Gette, I. F., Danilova, I. G., & Kovaleva, E. G. (2020). Anti-diabetic effect of isoflavone rich kudzu root extract in experimentally induced diabetic rats. *Journal of Functional Foods, 68*, Article 103922. https://doi.org/10.1016/j.jff.2020.103922.

Elsner, M., Guldbakke, B., Tiedge, M., Munday, R., & Lenzen, S. (2000). Relative importance of transport and alkylation for pancreatic beta-cell toxicity of streptozotocin. *Diabetologia*. https://doi.org/10.1007/s001250051564.

Fernandez, M. L., & West, K. L. (2005). Mechanisms by which dietary fatty acids modulate plasma lipids. *Journal of Nutrition*. https://doi.org/10.1093/jn/135.9.2075.

Fowler, M. J. (2008). Microvascular and macrovascular complications of diabetes. *Clinical Diabetes, 26*(2), 77–82. https://doi.org/10.2337/diaclin.26.2.77.

Freitas de Lima, F., Traesel, G. K., Menegati, S. E. L. T., Santos, A. C. dos, Souza, R. I. C., de Oliveira, V. S., … Vieira, M. do C. (2017). Acute and subacute oral toxicity assessment of the oil extracted from Attalea phalerata Mart ex Spreng. pulp fruit in rats. Food Research International, 91, 11–17. Doi: 10.1016/j.foodres.2016.11.019.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry, 18*(6), 499–502. https://doi.org/10.1093/clinchem/18.6.499.

Han, J. R., Deng, B., Sun, J., Chen, C. G., Corkey, B. E., Kirkland, J. L., … Guo, W. (2007). Effects of dietary medium-chain triglyceride on weight loss and insulin sensitivity in a group of moderately overweight free-living type 2 diabetic Chinese subjects. *Metabolism: Clinical and Experimental, 56*(7), 985–991. https://doi.org/10.1016/j.metabol.2007.03.005.

Henderson, A., Galeano, G., & Bernal, R. (1997). *Field guide to the palms of the Americas*. Princeton, NJ, USA: Princeton University Press.

Inocas. (2020). Innovative oil and carbon solutions. https://www.inocas.com.br/en/about/ Access Apr 2020.

International Diabetes Federation. (2019). IDF diabetes atlas - ninth edition 2019. https://idf.org/e-library/epidemiology-research/diabetes-atlas/159-idf-diabetes-atlas-ninth-edition-2019.

Kochikuzhyil, B. M., Devi, K., & Fattepur, S. R. (2010). Effect of saturated fatty acid-rich dietary vegetable oils on lipid profile, antioxidant enzymes and glucose tolerance in diabetic rats. *Indian Journal of Pharmacology, 42*(3), 142–145. https://doi.org/10.4103/0253-7613.66835.

Lescano, C. H., Iwamoto, R. D., Sanjinez-Argandoña, E. J., & Kassuya, C. A. L. (2015). Diuretic and anti-inflammatory activities of the microencapsulated *Acrocomia aculeata* (Arecaceae) oil on wistar rats. *Journal of Medicinal Food, 18*(6), 656–662. https://doi.org/10.1089/jmf.2014.0077.

Lieb, V. M., Schex, R., Esquivel, P., Jiménez, V. M., Schmarr, H. G., Carle, R., & Steingass, C. B. (2019). Fatty acids and triacylglycerols in the mesocarp and kernel oils of maturing Costa Rican *Acrocomia aculeata* fruits. *NFS Journal, 14*, 6–13. https://doi.org/10.1016/j.nfs.2019.02.002.

Magalhães, K. T., de Sousa Tavares, T., & Nunes, C. A. (2020). The chemical, thermal and textural characterization of fractions from Macauba kernel oil. *Food Research International, 130*, Article 108925. https://doi.org/10.1016/j.foodres.2019.108925.

Marinangeli, C. P. F., Varady, K. A., & Jones, P. J. H. (2006). Plant sterols combined with exercise for the treatment of hypercholesterolemia: Overview of independent and synergistic mechanisms of action. *Journal of Nutritional Biochemistry, 17*(4), 217–224. https://doi.org/10.1016/j.jnutbio.2005.09.003.

Marten, B., Pfeuffer, M., & Schrezenmeir, J. (2006). Medium-chain triglycerides. *International Dairy Journal, 16*(11), 1374–1382. https://doi.org/10.1016/j.idairyj.2006.06.015.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*(7), 412–419. https://doi.org/10.1007/BF00280883.

Montgomery, M. K., Osborne, B., Brown, S. H. J., Small, L., Mitchell, T. W., Cooney, G. J., & Turner, N. (2013). Contrasting metabolic effects of medium-versus long-chain fatty acids in skeletal muscle. *Journal of Lipid Research, 54*(12), 3322–3333. https://doi.org/10.1194/jlr.M040451.

Moreira, S. L. S., Pires, C. V., Marcatti, G. E., Santos, R. H. S., Imbuzeiro, H. M. A., & Fernandes, R. B. A. (2018). Intercropping of coffee with the palm tree, macauba, can mitigate climate change effects. *Agricultural and Forest Meteorology, 256*, 379–390. https://doi.org/10.1016/j.agrformet.2018.03.026.

Naskar, S., Mazumder, U. K., Pramanik, G., Gupta, M., Suresh Kumar, R. B., Bala, A., & Islam, A. (2011). Evaluation of antihyperglycemic activity of *Cocos nucifera* Linn. on streptozotocin induced type 2 diabetic rats. *Journal of Ethnopharmacology, 138*(3), 769–773. https://doi.org/10.1016/j.jep.2011.10.021.

Nevin, K. G., & Rajamohan, T. (2004). Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. *Clinical Biochemistry, 37*(9), 830–835. https://doi.org/10.1016/j.clinbiochem.2004.04.010.

Ngala, R. A., Ampong, I., Sakyi, S. A., & Anto, E. O. (2016). Effect of dietary vegetable oil consumption on blood glucose levels, lipid profile and weight in diabetic mice: An experimental case-control study. *BMC Nutrition, 2*(1), 1–8. https://doi.org/10.1186/s40795-016-0053-y.

Nunes, Â. A., Buccini, D. F., Jaques, J. A. S., Portugal, L. C., Guimarães, R. C. A., Favaro, S. P., … Carvalho, C. M. E. (2018). Effect of *Acrocomia aculeata* kernel oil on adiposity in type 2 diabetic rats. *Plant Foods for Human Nutrition, 73*(1), 61–67. https://doi.org/10.1007/s11130-017-0648-8.

Park, G. M., Lee, Y., Won, K. B., Yang, Y. J., Park, S., Ann, S. H., … Lee, S. G. (2019). High HDL-C levels reduce the risk of obstructive coronary artery disease in asymptomatic diabetics who achieved optimal glycemic control. *Scientific Reports, 9*(1), 1–7. https://doi.org/10.1038/s41598-019-51732-6.

Poznyak, A., Grechko, A. V., Poggio, P., Myasoedova, V. A., Alfieri, V., & Orekhov, A. N. (2020). The diabetes mellitus–atherosclerosis connection: The role of lipid and glucose metabolism and chronic inflammation. *International Journal of Molecular Sciences, 21*(5), 1–13. https://doi.org/10.3390/ijms21051835.

Priscilla, D. H., Jayakumar, M., & Thirumurugan, K. (2015). Flavanone naringenin: An effective antihyperglycemic and antihyperlipidemic nutraceutical agent on high fat diet fed streptozotocin induced type 2 diabetic rats. *Journal of Functional Foods, 14*, 363–373. https://doi.org/10.1016/j.jff.2015.02.005.

Pujol, J. B., Christinat, N., Ratinaud, Y., Savoia, C., Mitchell, S. E., & Dioum, E. H. M. (2018). Coordination of GPR40 and ketogenesis signaling by medium chain fatty acids regulates beta cell function. *Nutrients, 10*, 473. https://doi.org/10.3390/nu10040473.

Rangel, É. B., Rodrigues, C. O., & De Sá, J. R. (2019). Micro- and macrovascular complications in diabetes mellitus: Preclinical and clinical studies. *Journal of Diabetes Research, 2019*, 1–6. https://doi.org/10.1155/2019/2161085.

Reed, M. J., Meszaros, K., Entes, L. J., Claypool, M. D., Pinkett, J. G., Gadbois, T. M., & Reaven, G. M. (2000). A new rat model of type 2 diabetes: The fat-fed, streptozotocin-treated rat. *Metabolism - Clinical and Experimental, 49*(11), 1390–1394. https://doi.org/10.1053/meta.2000.17721.

Reeves, P. G., Nielsen, F. H., & Fahey, G. C. (1993). AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition*. https://doi.org/10.1093/jn/123.11.1939.

Schönfeld, P., & Wojtczak, L. (2016). Short- and medium-chain fatty acids in energy metabolism: The cellular perspective. *Journal of Lipid Research, 57*(6), 943–954. https://doi.org/10.1194/jlr.R067629.

Shinohara, H., Ogawa, A., Kasai, M., & Aoyama, T. (2005). Effect of randomly interesterified triacylglycerols containing medium- and long-chain fatty acids on energy expenditure and hepatic fatty acid metabolism in rats. *Bioscience, Biotechnology and Biochemistry, 69*(10), 1811–1818. https://doi.org/10.1271/bbb.69.1811.

Siddalingaswamy, M., Rayaorth, A., & Khanum, F. (2011). Anti-diabetic effects of cold and hot extracted virgin coconut oil. *Journal of Diabetes Mellitus, 1*(4), 118–123. https://doi.org/10.4236/jdm.2011.14016.

Soleá. (2020). Available at https://www.soleabrasil.com.br/ Access Apr 2020.

Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C. L., & Ramarao, P. (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacological Research, 52*(4), 313–320. https://doi.org/10.1016/j.phrs.2005.05.004.

St-Onge, M. P., Bourque, C., Jones, P. J. H., Ross, R., & Parsons, W. E. (2003). Medium-versus long-chain triglycerides for 27 days increases fat oxidation and energy expenditure without resulting in changes in body composition in overweight women. *International Journal of Obesity, 27*(1), 95–102. https://doi.org/10.1038/sj.ijo.0802169.

St-Onge, M. P., Ross, R., Parsons, W. D., & Jones, P. J. H. (2003). Medium-chain triglycerides increase energy expenditure and decrease adiposity in overweight men. *Obesity Research, 11*(3), 395–402. https://doi.org/10.1038/oby.2003.53.

St-Onge, M. P., & Bosarge, A. (2008). Weight-loss diet that includes consumption of medium-chain triacylglycerol oil leads to a greater rate of weight and fat mass loss than does olive oil. *The American Journal of Clinical Nutrition, 87*(3), 621–626. https://doi.org/10.1038/jid.2014.371.

Subramanian, P., Jayakumar, M., Singaravel, M., Kumar, D., Basu, P., Jayapalan, J. J., & Hashim, O. H. (2015). Fisetin, a dietary flavonoid, attenuates hyperammonemia and improves circadian locomotor deficits, redox balance, and astrocytic markers in rats. *Journal of Functional Foods, 12*, 409–419. https://doi.org/10.1016/j.jff.2014.11.025.

Subramanian, S. P., & Prasath, G. S. (2014). Antidiabetic and antidyslipidemic nature of trigonelline, a major alkaloid of fenugreek seeds studied in high-fat-fed and low-dose streptozotocin-induced experimental diabetic rats. *Biomedicine and Preventive Nutrition, 4*(4), 475–480. https://doi.org/10.1016/j.bionut.2014.07.001.

Sung, M. H., Liao, F. H., & Chien, Y. W. (2018). Medium-chain triglycerides lower blood lipids and body weight in streptozotocin-induced type 2 diabetes rats. *Nutrients, 10*(8), 963. https://doi.org/10.3390/nu10080963.

Tilahun, W. W., Grossi, J. A. S., Favaro, S. P., Sediyama, C. S., Goulart, S. D. M., Pimentel, L. D., & Motoike, S. Y. (2019). Increase in oil content and changes in quality of macauba mesocarp oil along storage. *OCL - Oilseeds and Fats, Crops and Lipids, 26*(20), 8. https://doi.org/10.1051/ocl/2019014.

Turner, N., Hariharan, K., TidAng, J., Frangioudakis, G., Beale, S. M., Wright, L. E., … Ye, J. M. (2009). Enhancement of muscle mitochondrial oxidative capacity and alterations in insulin action are lipid species dependent: Potent tissue-specific effects of medium-chain fatty acids. *Diabetes, 58*(11), 2547–2554. https://doi.org/10.2337/db09-0784.

Veerapur, V. P., Prabhakar, K. R., Kandadi, M. R., Srinivasan, K. K., & Unnikrishnan, M. K. (2010). Antidiabetic effect of *Dodonaea viscosa* aerial parts in high fat diet and low dose streptozotocin-induced type 2 diabetic rats: A mechanistic approach. *Pharmaceutical Biology, 48*(10), 1137–1148. https://doi.org/10.3109/13880200903527736.

von Hanstein, A. S., Lenzen, S., & Plötz, T. (2020). Toxicity of fatty acid profiles of popular edible oils in human EndoC-βH1 beta-cells. *Nutrition and Diabetes*. https://doi.org/10.1038/s41387-020-0108-7.