

Full Length Research Paper

The medicinal species *Myracrodruon urundeuva* Fr. All. presents antidiabetic and hypolipidemic properties, probably related to its antioxidant and anti-inflammatory actions

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The Type 2 diabetes (T2D) is a global epidemics and considered as an inflammation related disease. *Myracrodruon urundeuva* (Anacardiaceae) is used in Northeast Brazil, because of its anti-inflammatory properties. The species is known to present tannins and chalcones among its active compounds. The objectives of the work were to investigate the antidiabetic and hypolipidemic activities of the stem bark decoction (MUSB) from a cultivated specimen of *M. urundeuva*, in the alloxan-induced diabetes model. For that, male Wistar rats were divided into normal controls, untreated and treated (MUSB, 50 and 100 mg/kg, 7 days) diabetic groups. Afterwards, the animals were subjected to biochemical measurements (glycemia, cholesterol, triglycerides and ALT and AST liver transaminases) before (48 h, post-alloxan) and after treatments, then euthanized for histopathological studies. The data were analyzed by ANOVA and Tukey's test and considered significant for $p < 0.05$. We observed significant decreases in glycemia, cholesterol and triglycerides values. ALT and AST values were within normal ranges. The histopathological examination revealed changes of somehow less intensity in treated diabetic, relatively to diabetic animals without treatment. An interesting finding was the beta cell proliferation observed in diabetic pancreas after MUSB treatments. The antidiabetic and hypolipidemic effects are possibly related to the anti-inflammatory and antioxidant actions of MUSB. The regulation of beta cell mass, as a strategic target for T2D treatment, should stimulate translational studies dealing with the effects of *M. urundeuva* on beta cell proliferation.

Key words: *Myracrodruon urundeuva*, antidiabetic and hypolipidemic effects, beta cell proliferation

INTRODUCTION

Type 2 diabetes (T2D) is a metabolic disorder characterized by the presence of hyperglycemia, due to defective insulin secretion and/or defective insulin action. The chronic hyperglycemia is associated with long-term microvascular complications, as well as an increased risk of cardiovascular disease. According to the Global Report on Diabetes, from the World Health Organization (WHO), 2016 [1], the number of cases and the prevalence of T2D have been increasing over the past few decades. An estimated 422 million adults were living with diabetes, in 2014, and the global prevalence, since 1980, rised from 4.7% to 8.5% in the adult population. This reflects an increase in associated risk factors, such as overweight or obesity. Diabetes prevalence has risen faster in low- and middle-income countries than in high-income countries.

The causes of increasing T2D are embedded in a very complex group of genetic and epigenetic systems that act within an equally complex framework determining behavior and also with environmental influences [2]. Furthermore, T2D presents significant social and economic burdens related to complications that account for increased morbidity and mortality. Inflammatory response likely contributes to T2D occurrence by causing insulin resistance and is, in turn, intensified in the presence of hyperglycemia to promote long-term complications. Thus, targeting inflammatory pathways could possibly be a component of strategies to prevent and control diabetes and related complications.

Furthermore, insufficient insulin secretion by pancreatic beta cells, in order to compensate for insulin resistance, is a fundamental cause of diabetic hyperglycemia [3,4], making beta cells a central player in the pathogenesis of diabetes [5]. Thus, beta cell dysfunction and decreased beta cell mass are crucial to diabetes development, shifting the goal for diabetes treatment from merely reducing glucose concentrations to preventing decline in beta cell function [6]. Although obesity, dyslipidemia and insulin resistance are important risk factors for T2D development, the major factor is beta cell failure [7].

Myracrodruon urundeuva Fr. All. belongs to the Anacardiaceae family and is widely used in popular medicine, in the Northeastern region of Brazil (where it is known as "aroeira-do-sertão"), mainly in the treatment of inflammation-related conditions. Importantly, *M. urundeuva* is one of the medicinal species most used in that semi-arid region of Brazil [8,9,10]. Previously [11,12,13,14] we showed that the *M. urundeuva* anti-inflammatory activities are probably due to the presence of phenol compounds, as tannins and chalcones, among others.

Lately, we demonstrated that this species also protects mesencephalic cells against 6-OHDA-induced neurotoxicity and reduces dopaminergic loss, in a model of Parkinson's disease in rats [15,16]. This effect, in turn,

emphasizes the potential importance of *M. urundeuva* as a therapeutic strategy in the treatment of neurological conditions, as neurodegenerative disorders where inflammation is also involved.

Furthermore, a subclinical inflammatory reaction has been shown to precede the onset of T2D and to have an important role in the destruction of pancreatic beta cells, leading to T1D [17]. In addition, evidences indicate that the pattern of circulating inflammatory cytokines modifies the risk for T2D. Thus, the elevation of IL-1 β and IL-6 alone or combined increases the risk for this disorder [18]. Moreover, a better understanding of inflammatory mechanisms involved with T2D may provide newer therapeutic strategies for the treatment of this disease.

Thus, considering the association of T2D with inflammation and the potent anti-inflammatory properties of *M. urundeuva*, the objectives of the present work were to evaluate the possible antidiabetic effects of this species. For that, we measured blood biochemical parameters (glycemia, total cholesterol and triglycerides) of diabetic rats subjected to the alloxan-induced diabetes. Besides, histological analyses of pancreas, liver and kidney were also carried out.

MATERIAL AND METHODS

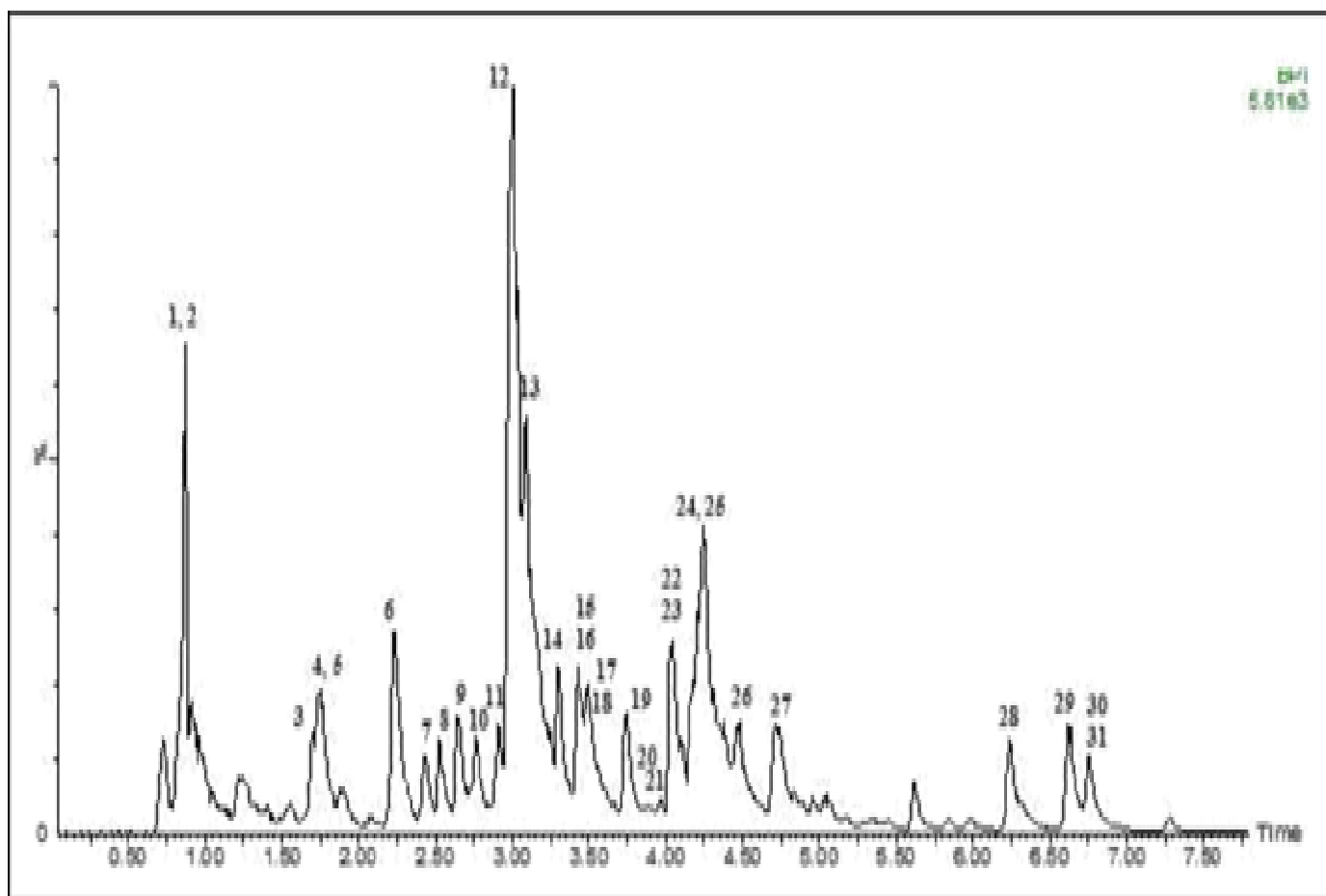
2.1. Drugs. Alloxan was purchased from Sigma-Aldrich (MO, USA). All the kits for measurements of biochemical parameters were from Labtest Diagnóstica S.A. (Lagoa Santa, MG, Brazil). Antibodies for immunohistochemistry assays were from Santa Cruz Biotechnology (Dallas, TX, USA) or Merck-Millipore (Darmstadt, Germany). All other reagents were of analytical grade.

2.2. Animals. Male Wistar rats (200-250 g) from the Animal House of the Faculty of Medicine Estácio of Juazeiro do Norte (Estácio/FMJ), Ceará, Brazil, were divided into the following groups: normal controls (administered with distilled water), untreated diabetic (administered with distilled water) and diabetic treated with the *M. urundeuva* stem bark decoction (MUSB), at the doses of 50 and 100 mg/kg, p.o., daily for 7 days. The project was approved by the Institutional Ethics Committee on Animal Experimentation of the Estácio/FMJ (2014.1-004). The experiments were performed according to the Guide for the Care and Use of Laboratory Animals (NIH, USA, 2011).

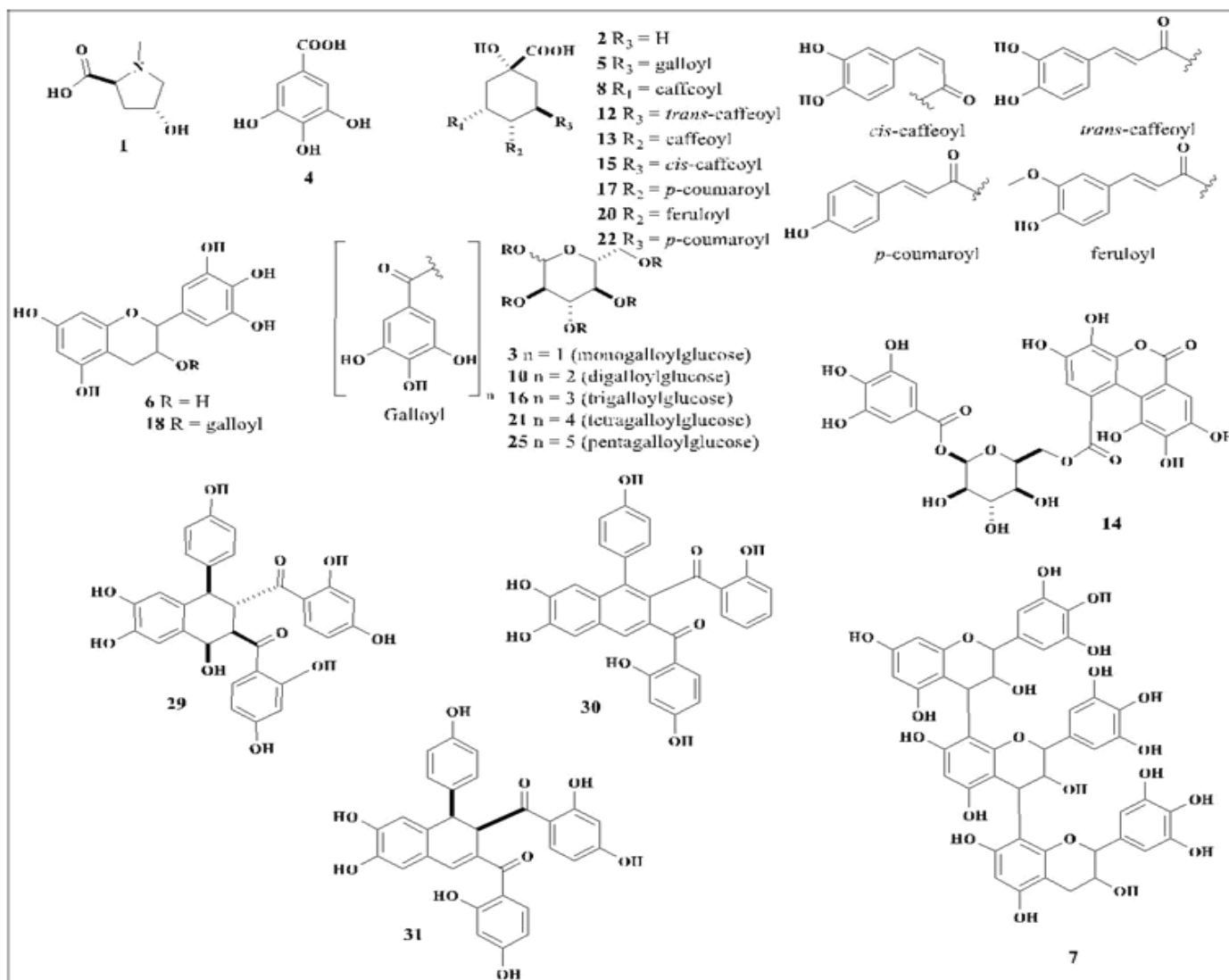
2.3. Plant material. The seeds from wild specimens of *M. urundeuva* were cultivated in the horticulture sector of the Federal University of Ceará (UFC, Fortaleza, Brazil), in February 2012, and the exsiccatae are deposited at the UFC Prisco Bezerra Herbarium, under the number 48,904. The stem bark of a cultivated specimen of the *E*- β -ocimene chemotype [19] was obtained from a 7-year-old plant. For the extraction, five aliquots (200 g each) of

this *M. urundeuva* stem bark were triturated after drying at room temperature and, then, submitted to decoction for 15 min, in 900 mL water (3x), yielding the decoction liquid that was lyophilized to yield 210.5 g of a solid residue designated MUSB. The analysis was made with an Acquity UPLC system coupled to a quadrupole/time-of-flight system (both: Waters, Milford, MA, USA). MUSB was chromatographed on an Acquity BEH C18 (1.7 μ m, 2.1 \times 150 mm; also: Waters, Milford, MA, USA) column at 40°C. The identification of the compounds present in the stem bark of the decoction was carried out by comparison of the mass spectrometric data, obtained

under both negative and positive electrospray ionization conditions, with the data available in the literature. The correspondent peak of each compound was numbered after its elution order, identified and classified into seven groups: non-proteic amino acids (one compound: **1**), carboxylic acids (three compounds: **2**, **4** & **5**), catechins (two compounds: **6** & **18**), chlorogenic and cinnamic acid derivatives (seven compounds: **8**, **12**, **13**, **15**, **17**, **20** & **22**), hydrolysables tannins (six compounds: **3**, **10**, **14**, **16**, **21** & **25**), condensed tannins (eight compounds: **7**, **9**, **11**, **19**, **23**, **24**, **26** & **27**) and dimeric chalcones (four compounds: **28**, **29**, **30** & **31**).



UPLC-ESI-QTOF-MS chromatogram of the decoction from the stem bark of a cultivated specimen of *Myracrodruon urundeuva* (MUSB).



Proposed structures for the identified compounds of the decoction from the stem bark of a cultivated specimen of *Myracrodruon urundeuva*.

2.4. Alloxan-induced diabetes model. The cytotoxic action of alloxan is mediated by reactive oxygen species (ROS) which enters the beta cells, via a glucose transporter (GLUT-2). The action of ROS and the massive increase in cytosolic calcium concentration cause beta cells destruction [20, 21]. Moreover, the excess generation of ROS due to hyperglycemia causes oxidative stress, further exacerbating the development and progression of diabetes and its complications [22]. For the diabetic groups, the animals were intravenously injected (penile vein) with the alloxan solution (40-50 mg/kg). The animals had their blood collected from the retroorbital plexus, 48 h after the alloxan injection (when treatments started) and 7 days later, for biochemical measurements.

2.5. Measurements of biochemical parameters. For the determination of sera glucose, cholesterol, triglycerides and liver transaminases (AST and ALT), the

animals from untreated and treated diabetic groups were injected with alloxan and, 48 h later, these animals as well as nondiabetic ones had their blood collected (1 mL) from the retro-orbital plexus, under light anesthesia, for biochemical measurements. This was the experimental zero-day (T0). Then, the treatments started, lasting for 7 days. At the 7th day (T7), the animals had their blood collected again for a new biochemical determination. All conventional laboratory measurements were done according to the manufacturer's instructions (Labtest Diagnóstica S.A.).

2.6. Histological analyses. After 7-day treatments, the animals were euthanized and pancreas, liver and kidneys from all groups were dissected, fixed in 10% buffered formalin, followed by immersion in a 70% alcohol solution and embedded in paraffin. These paraffin blocks were used for the preparation of 6 μm sections and hematoxylin-eosin staining. Slides were

examined by optic microscopy, with x100 and x400 magnifications.

2.7. Immunohistochemical assays. After 7-day treatments, the animals were euthanized and the pancreases from all groups were dissected, fixed in 10% buffered formalin, followed by immersion in a 70% alcohol solution for 24 h and embedded in paraffin. The paraffin blocks were used for the preparation of 6 μm sections and immunohistochemical assays. For that, the sections were deparaffinized, dehydrated in xylol and ethanol, and immersed in 0.1 M citrate buffer (pH 6) under microwave heating for 18 min, for antigen recovery. After cooling at RT for 20 min, the sections were washed in PBS, followed by a 15 min blockade of the endogenous peroxidase with a 3% H₂O₂ solution. The sections were incubated overnight at 4°C with rabbit primary antibodies (anti-iNOS or anti-COX-2) diluted in PBS-BSA, according to the manufacturers' instructions. At the next day, the sections were washed in PBS and incubated for 30 min with the secondary biotinylated rabbit antibody (anti-IgG, 1:200 dilution) in PBS-BSA. After washing in PBS, the sections were incubated for 30 min with the conjugated streptavidin peroxidase complex (ABC Vectastain® complex, Vector Laboratories, Burlingame, CA, USA). After another washing with PBS, the sections were stained with 3,3'-diaminobenzidine-peroxidase (DAB) chromophore, counter-stained with Mayer hematoxylin, dehydrated and mounted in microscope slides for analyses.

2.8. Statistical analyses. The data were presented as means \pm SEM. The immunohistochemistry data were semi-quantified by the Image J software (NIH, USA) and all data were analyzed by One-way ANOVA, followed by Tukey as the *post hoc* test and considered significant for $p < 0.05$.

RESULTS

3.1. Blood glycemia. While no changes in blood glucose were observed in diabetic animals, at T₀ in relation to T₇, 44 and 38% reductions were demonstrated in diabetic animals after a 7-day treatment with MUSB 50 and 100 mg/kg, respectively, related to each group at T₀ (Figure 1).

3.2. Blood cholesterol and triglycerides. Similar decreases (around 35%) were observed in cholesterol values, after 7-day treatments with MUSB, at both doses used (Figure 2). Higher decreases (74 and 75%) were observed in triglycerides (TG) levels from diabetic animals, after MUSB treatments with the doses of 50 and 100 mg/kg, respectively, in relation to T₀ of each group (Figure 3).

3.3. Blood liver transaminases. Although, for all groups tested (diabetic rats untreated and treated with

MUSB, 50 and 100 mg/kg) there was an increase in concentrations of both AST and ALT, these changes were in the range of reference values for the animal species used in the present work (Figures 4 and 5).

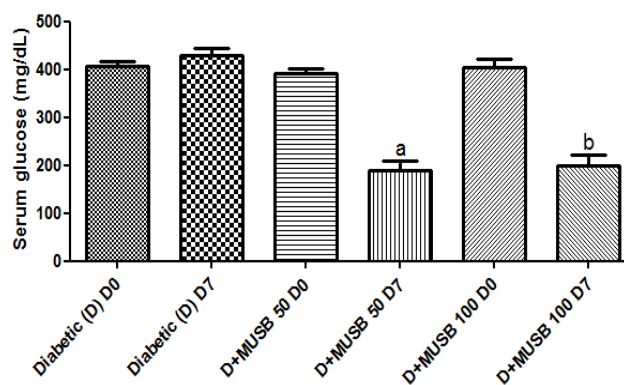


Figure 1: *Myracrodruon urundeuva* decoction (MUSB) treatments, for 7 days, decreases blood glucose levels in alloxan-induced diabetic rats (D). a. vs. D+MUSB-50 T₀, $q=11.49$, $p < 0.001$; b. vs. D+MUSB-100 T₀, $q=9.702$, $p < 0.001$. T₀= 48 h after alloxan-induced diabetes (One way ANOVA and Tukey as the *post hoc* test).

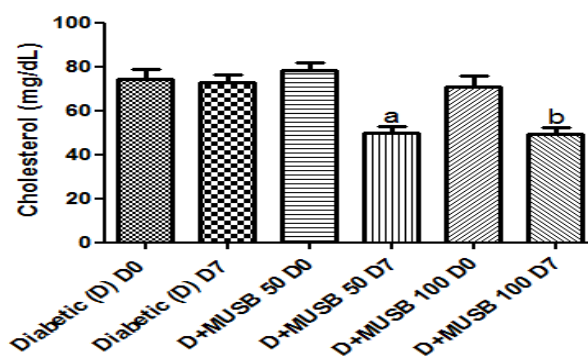


Figure 2: *Myracrodruon urundeuva* decoction (MUSB) treatments, for 7 days, decreases blood cholesterol levels in alloxan-induced diabetic rats (D). a. vs. D+MUSB-50 T₀, $q=6.865$, $p < 0.001$; b. vs. D+MUSB-100 T₀, $q=5.477$, $p < 0.01$. T₀=48 h after alloxan-induced diabetes (One way ANOVA and Tukey as the *post hoc* test).

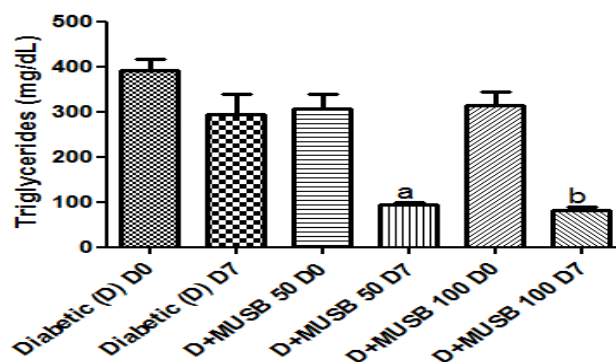


Figure 3: *Myracrodruon urundeuva* decoction (MUSB) treatments, for 7 days, decreases blood triglycerides levels in alloxan-induced diabetic rats (D). a. vs. D+MUSB-50 T₀, $q=9.467$, $p < 0.001$; b. vs. D+MUSB-100 T₀, $q=10.37$, $p < 0.001$. T₀=48 h after alloxan-induced diabetes (One way ANOVA and Tukey as the *post hoc* test).

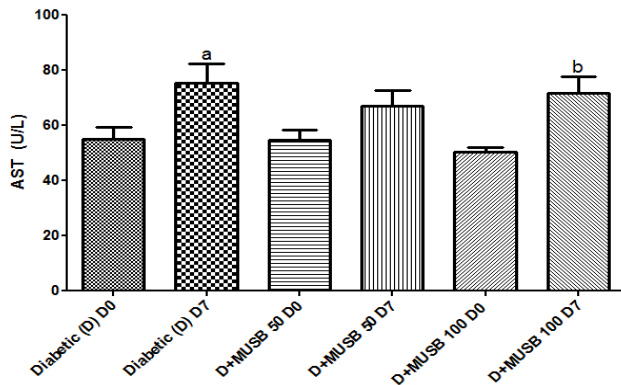


Figure 4: *Myracrodruon urundeuva* decoction (MUSB) treatments, for 7 days, did not reverse the increase in AST levels observed in the alloxan-induced diabetic rats (D). **a.** vs. Diabetic (D) T0, $q=4.121$, $p<0.05$; **b.** vs. D+MUSB-100 T0, $q=4.308$. T0=48 h after alloxan-induced diabetes (One way ANOVA and Tukey as the *post hoc* test).

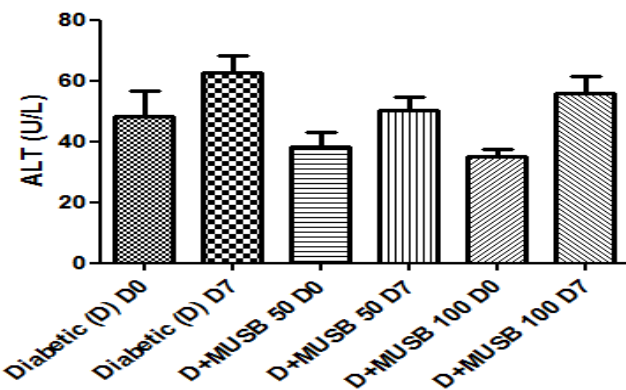


Figure 5: *Myracrodruon urundeuva* decoction (MUSB-100) treatments, for 7 days, did not alter ALT levels observed in the alloxan-induced diabetic rats, D (One way ANOVA and Tukey as the *post hoc* test).

3.4. Histopathological analyses (HE staining).

Pancreas from controls showed preserved acini and normal islets, while diabetic rats presented pancreatic islets with atresia and reduced size. Interestingly, the diabetic animals after treatment with MUSB, at both doses (50 and 100 mg/kg), showed pancreatic islets with a proliferative aspect (Figure 6). The liver microscopic analyses of the control group showed, in general, a normal histological pattern, but also some perivascular inflammatory infiltrate and congested vessels, while the diabetic group showed congested vessels, interstitial edema, sinusoidal dilatation and inflammatory infiltration. The diabetic animals after treatment with MUSB, at the lower dose (50 mg/kg), sometimes showed congested vessels, sinusoidal dilatation, interstitial edema and inflammatory infiltrate. However, normal lobuli and septa were usually observed, as well as a normal portal triad and normal hepatocytes. The 100 mg/kg-treated group showed congested vessels and interstitial hemorrhage, sinusoidal dilatation and inflammatory infiltrate. All these

liver alterations after the MUSB treatments were however of less intensity, in relation to the diabetic liver

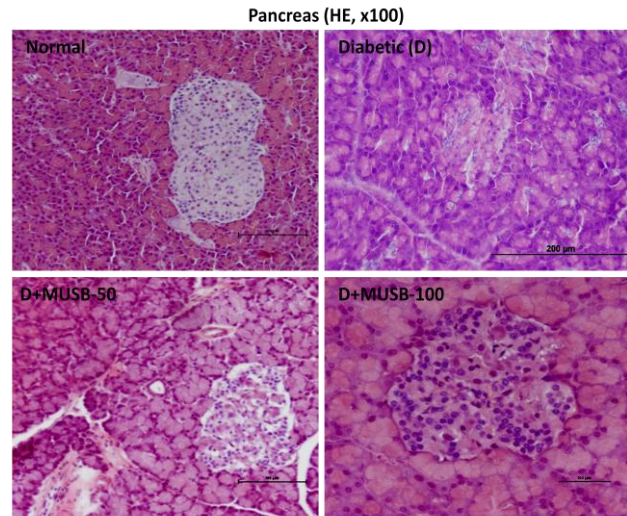


Figure 6: Representative photomicrographs (HE, x400) of normal and alloxan-induced diabetic rat pancreas, untreated (D) or treated with MUSB (50 and 100 mg/kg, p.o., 7 days). The untreated diabetic pancreas (D) shows atresic and congested islets with reduced size. In diabetic rat pancreas after MUSB treatments, islets of Langerhans appear with proliferative characteristics. T0=48 h after diabetes induction and T7=7 days latter (Nikon Eclipse Nis, Software Nis 4.0, scale=100 and 200 μ m).

without treatment (Figure 7). While kidneys from the control group showed normal features, the diabetic group presented tubular tumefaction, interstitial and intratubular hemorrhage, congested interstitial capillary, vacuolar degeneration and tubular cells degeneration. The diabetic group treated with MUSB, at the dose of 50 mg/kg, showed interstitial hemorrhage, congested glomerular capillaries and vacuolized cells of the dense macula detaching from the basal membrane, small cytoplasmic vacuoles in the proximal contorted tubules, discrete inflammatory infiltrate and a discontinuous tubular epithelium. A similar pattern, although with less changes, was observed in diabetic rats after MUSB treatment, at the dose of 100 mg/kg. Thus, the kidneys showed vacuolar and glomerular degeneration with discontinuous Bowman capsule cells, intratubular proteic material deposition, interstitial hemorrhage, edema and vacuole and tubular degeneration (Figures 8A and 8B).

3.5. Immunohistochemistry analyses for iNOS and COX-2 in rat pancreas.

We showed almost a 4-times increase in iNOS immunoreactivity in the pancreas from diabetic animals, compared with the normal control group (Figure 9A). The MUSB treatment of diabetic animals caused a partial or complete recovery, after the doses of 50 and 100 mg/kg, respectively. An increase of 2.8-times was also observed in COX-2 immunoreactivity in diabetic pancreas and this value was not different

from the normal controls, after MUSB treatments with both doses (Figure 9B).

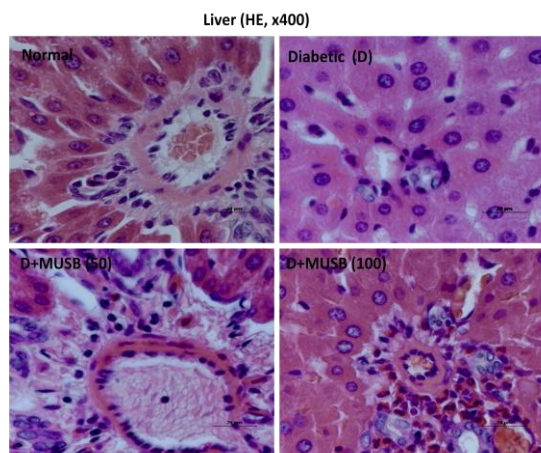


Figure 7: Representative photomicrographs (HE, x400) of normal and alloxan-induced diabetic rat liver, untreated (D) or treated with MUSB (50 and 100 mg/kg, p.o., 7 days). In the untreated diabetic liver, there are inflammatory cells in the space of Disse and hepatocytes with pyknotic nuclei. After MUSB-50 and MUSB-100 treatments inflammatory infiltrates with mast cells and lymphocytes were still observed (Nikon Eclipse Nis, Software Nis 4.0, scale=20 μ m).

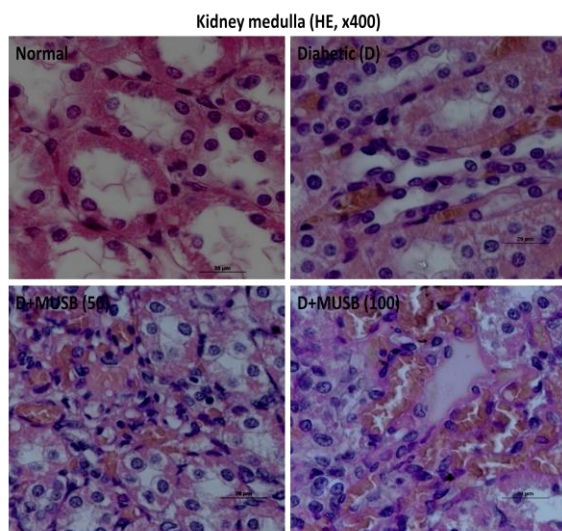


Figure 8: Representative photomicrographs (HE, x400) of normal and alloxan-induced diabetic rat kidney, untreated (D) or treated with MUSB (50 and 100 mg/kg, p.o., 7 days). **A. renal medulla:** In the untreated diabetic kidney, there are hemorrhage, distal convoluted tubule with tumefaction and vacuolar degeneration of epithelial cells. After MUSB treatments, interstitial hemorrhage and hydropic degeneration of renal tubular epithelium are shown. **B. renal cortex:** In the untreated diabetic kidney, there are interstitial congested vessels. After MUSB treatments, there are glomeruli tumefaction, vacuolar degeneration and intraglomerular hemorrhage (Nikon Eclipse Nis, Software Nis 4.0, scale=20 μ m).

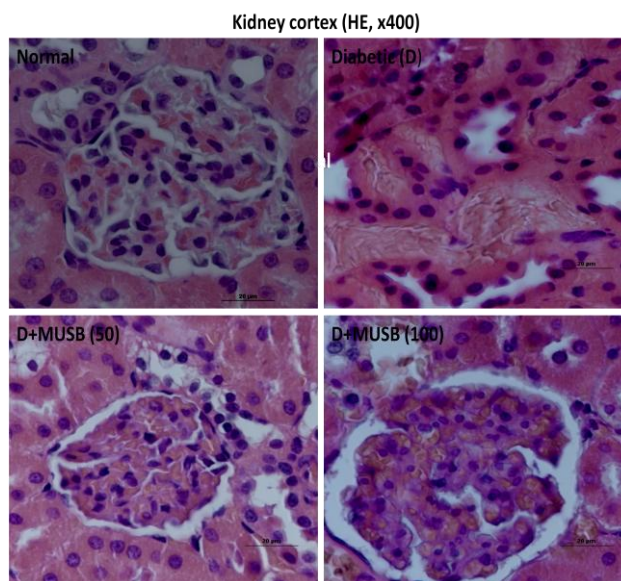


Figure 9: *Myracrodruon urundeuva* (MUSB-100) treatments, for 7 days, decreases iNOS and COX-2 immunoreactivity in pancreas from alloxan-induced diabetic rats (D). **iNOS:** **a.** vs. normal controls, $q=18.08$, $p<0.001$; **b.** vs. D+MUSB 50, $q=9.379$; $p<0.001$; **c.** vs. D+MUSB 100, $q=15.31$, $p<0.001$; **d.** vs. normal controls, $q=7.363$; $p<0.01$; **e.** vs. D+MUSB 100, $q=5.553$, $p<0.05$. **COX-2:** **a.** vs. normal controls, $q=22.66$, $p<0.001$; **b.** vs. D+MUSB 50, $q=26.28$, $p<0.001$; **c.** vs. D+MUSB 100, $q=26.13$, $p<0.001$ (One way ANOVA and Tukey as the *post hoc* test).

DISCUSSION

Type 2 diabetes (T2D), the most common form of the disease, is characterized by insufficient secretion of insulin from pancreatic beta-cells, coupled with impaired insulin action in muscles, liver and adipose tissue, named as insulin resistance. The impaired insulin secretion could be due to a decline in beta-cells secretory rate or to a decrease in beta-cell mass or both [23]. The increasing prevalence of T2D throughout the world has had a major impact on the development of diabetic kidney disease, one of the most frequent complications of diabetes [24]. Besides, renal complications are an important contributor to mortality in patients with T2D [25].

Alloxan-induced diabetes is known to trigger liver morphological and ultrastructural changes that closely resemble human disease, ranging from steatosis to steatohepatitis and liver fibrosis [26]. The liver contributes to maintain normal blood glucose concentration in the fasting and postprandial states. The loss of insulin effect in the liver leads to glycogenolysis and increased glucose production. Abnormalities of liver triglycerides storage and lipolysis are an early manifestation of insulin resistance and mild chronic elevations of liver transaminases often reflect insulin resistance [27].

Diabetes is mainly due to oxidative stress increasing reactive oxygen species that can have major effects. Many plants contain different natural antioxidants, in particular tannins, flavonoids, C and E vitamins, that have the ability to maintain β -cells performance and to decrease glucose levels in the blood. Both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. This oxidative stress leads to β -cell destruction by apoptosis. The antidiabetic properties of several medicinal species are mediated through their antioxidant and/or antiapoptotic properties in a streptozotocin induced stress model [28]. Furthermore, diabetes induces oxidative stress in the liver, that is characterized by increased concentration of reactive oxygen species (ROS) in the tissue and significant reduction in its antioxidant defenses. Such oxidative unbalance in liver cells may play a relevant role in the genesis of the chronic liver disease associated with diabetes [29].

In the present work, we showed for the first time the hypoglycemic effect of *M. urundeuva* in the model of alloxan-induced diabetes in rats. The aqueous extract (decoction, MUSB) was administered orally for 7 days, at the doses of 50 and 100 mg/kg. This medicinal species is well known for its anti-inflammatory properties, as evaluated in acute models of inflammation [11,12] and these effects seem to be, at least partly, related to inhibition of pro-inflammatory cytokines, as TNF- α [16]. Interestingly, *M. urundeuva* belongs to the Anacardiaceae family, as *Anacardium occidentale* and *Mangira indica*, medicinal species known for their antidiabetic activity [30, 31, 32, 33].

Furthermore, *M. urundeuva*, at the present study both doses, significantly decreased total cholesterol and triglyceride contents in the serum of diabetic animals, after a 7-day treatment. Cholesterol is important to health, but can be harmful when its levels are too high, contributing to the narrowing of arteries. Unfortunately, people with diabetes are more prone to having unhealthy high cholesterol levels, what contributes to cardiovascular disease. In addition, triglycerides are the most common type of fat in the body. A high triglyceride level, combined with low HDL cholesterol or high LDL cholesterol, is associated with atherosclerosis and the build up of fatty deposits in artery walls that increase the risk for heart attack, peripheral artery disease and stroke [34].

Diabetes is associated with a high risk of atherosclerosis and cardiovascular disease and, thus, it is the leading cause of death among patients with type 2 diabetes. T2D lipid abnormalities are defined by a high concentration of TG and a low concentration of HDL cholesterol [34]. Abnormalities in lipid metabolism in T2D are among the major factors contributing to an increased cardiovascular risk. The primary quantitative lipoprotein abnormalities are increased TG levels and decreased HDL-cholesterol levels [35]. Thus, the treatment of dyslipidemia in insulin resistant patients with T2D has

been successful in reducing cardiovascular disease (CVD). CVD is a major cause of morbidity and mortality in T2D patients, with two- to four-fold increases, when compared with non-diabetic individuals. Thus, LDL cholesterol, TG, and HDL cholesterol are all appropriate targets for therapy [36], pointing out the importance of new and safer therapeutic strategies.

In the present study, the histopathological changes observed in pancreas, liver and kidneys from diabetic rats, after MUSB treatments, were in general of less intensity, in relation to those of diabetic groups without treatment. Interestingly, while diabetic rats presented pancreatic islets with atresia and reduced size, diabetic animals after MUSB treatments showed beta-cells from pancreatic islets in a proliferative state.

The regulation of beta-cell mass represents a critical issue for understanding diabetes, a disease characterized by a near-absolute (T1D) or relative (T2D) deficiency in the number of pancreatic beta-cells. The low capacity for self-replication in the adult is limited and does not result in significant regeneration, following extensive tissue injury. Thus, chronically increased metabolic demands also can lead to beta-cell failure. Furthermore, the stimulation of beta-cell replication, inhibition of beta-cell apoptosis, and induction of beta-cell neogenesis are probably the best options for pharmacological restoration of a functional beta-cell mass, in diabetes. Pharmacological regulation of the beta-cell mass, therefore, represents another interesting target for the treatment of T2D patients [37].

A growing body of evidence [38- 43] points out to the importance of beta-cell mass to T2D development and progression. Beta-cell dysfunction in T2D patients might be induced by beta-cell loss and/or its functional defects. A very important point is that most people with insulin resistance will never develop T2D, which only results if the patients beta-cells fail to provide sufficient insulin [42]. Furthermore, the production of interleukin-1 β by beta-cells have been linked to beta-cells death in T2D [44].

Chronic inflammation is implicated in the pathogenesis of insulin resistance and iNOS is involved with many diseases associated with inflammation. Evidences suggest that iNOS plays a role in fasting hyperglycemia and contributes to hepatic insulin resistance in obese mice [45]. Furthermore, iNOS is an important regulator of insulin resistance in T2D and the understanding of molecular mechanisms regulating the iNOS pathway, under normal and hyperglycemic conditions, would explain some abnormalities observed in T2D [46]. We showed an almost 4-times increase in iNOS immunoreactivity in pancreas from diabetic animals, compared with the normal control. These values were significantly decreased in diabetic animals after MUSB treatments. Interestingly, iNOS seems to be involved with the regulation of beta-cell mass [47] and we also showed that the MUSB treatment leads to beta-cell proliferation.

COX-2 is upregulated in a variety of pathological conditions, including diabetes, and this event is associated with activation of downstream inflammatory reactions [48, 49]. Earlier [50], the administration of a selective COX-2 inhibitor was shown to prevent the onset of diabetes in mice. We observed an almost 3-times increase in COX-2 immunoreactivity in the diabetic pancreas, in relation to normal controls, and this increase was completely reversed after MUSB treatments. This could be justified by the anti-inflammatory effects of *M. urundeuva*, as earlier demonstrated by us [11, 12].

Diabetes is a global health problem and an economic burden. Although several antidiabetic drugs are available, the need for novel therapeutic agents with improved efficacy and few side-effects remains. Drugs derived from natural compounds are more attractive than synthetic drugs, because of their diversity and minimal side-effects. Published data suggest that natural compounds directly enhance insulin secretion, prevent pancreatic beta-cell apoptosis and modulate pancreatic beta-cell differentiation and proliferation [51]. Thus, it is essential to continuously investigate natural compounds as sources of novel pharmaceuticals, and to investigate their mechanisms of action, seeking the development of potential and safer antidiabetics.

Many plants are natural antioxidants and effective herbal medicines, in part due to their antidiabetic compounds, such as flavonoids, tannins, phenolics, and alkaloids, that improve the performance of pancreatic tissues by increasing the insulin secretion or decreasing the intestinal absorption of glucose and tannins. Phenolic compounds are present in *M. urundeuva* stem bark. Furthermore, natural and synthetic chalcones, as well, show antidiabetic and hypolipidemic effects [52-55]. In the present study, we showed that, by causing beta-cell proliferation and presenting an anti-inflammatory activity, *M. urundeuva* is a potential candidate to be considered in translational studies for the adjuvant treatment of T2D.

Conflict of interests

The authors declare no conflict of interests.

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