Original Paper

Anti-Diabetic Effects of the Ethyl-Acetate Fraction of *Trichilia catigua* in Streptozotocin-Induced Type 1 Diabetic Rats

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Key Words

Diabetes • Nephropathy • Pancreatic islets • β -cell • Antioxidants • *Trichilia catigua*

Abstract

Background/Aims: *Trichilia catigua* A. Juss., known as “catuaba” in Brazil, has been popularly used as a tonic for fatigue, impotence and memory deficits. Previously, our group demonstrated that the ethyl-acetate fraction (EAF) of *T. catigua* has antioxidant and anti-inflammatory effects. The present study evaluated the anti-diabetic activity of EAF in type 1 diabetic rats.

Methods: Male Wistar rats were divided into four groups (N: non-diabetic group, D: type 1 diabetic group, NC: non-diabetic + EAF group and DC: type 1 diabetic + EAF group). The latter two groups were treated with 200 mg/kg EAF. Type 1 diabetes was induced by intravenous streptozotocin (STZ) injection (35 mg/kg). Starting two days after STZ injection, EAF was administered daily by gavage for 8 weeks. **Results:** EAF attenuated body mass loss and reduced food and water intake. EAF improved hyperglycaemia and other biochemical parameters, such as alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Furthermore, the number of pancreatic β -cells and the size of the islets had increased by β -cell proliferation in the DC group. EAF promoted reduction in kidney tissue damage in STZ-induced diabetic rats by reduction of renal fibrosis. **Conclusion:** The present study showed that EAF improves glucose homeostasis and endocrine pancreas morphology and inhibits the development of diabetic nephropathy in STZ-induced diabetic rats.

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Introduction

Diabetes mellitus (DM) is a chronic non-communicable disease that results in chronic hyperglycaemia. Type 1 DM is characterized by pancreatic β -cell apoptosis and massive reduction in the number and area of the pancreatic islets [1-3]. In 2015 alone, this pathology affected 415 million people worldwide, caused 5.0 million deaths and cost 673 billion dollars from public coffers worldwide [4].

Chronic hyperglycaemia leads to elevated production of advanced glycation end products (AGEs) and oxidative/inflammatory activities. In this way, DM is associated with tissue injuries leading to a risk of cardiometabolic diseases and their related complications as long-term consequences, which include ketoacidosis, neuropathy, retinopathy, cardiovascular disorders and nephropathy [5].

Natural products with antioxidant activity have been used for the treatment of DM and diabetic nephropathy [6-8]. In addition, polyphenolic compounds, flavonoids, tannins, anthocyanins and other phenolic constituents prevent oxidative damage and several degenerative diseases [9, 10]. *Trichilia catigua* A. Juss., known as “catuaba” in Brazil, has been popularly used as a tonic for fatigue, impotence and memory deficits. It has been demonstrated that the ethyl-acetate fraction (EAF) of *T. catigua* has antioxidant and anti-inflammatory effects [10-12].

Previously, our group reported the antioxidant capacity of EAF from the bark of *T. catigua*, where nine polyphenolic constituents with antioxidant effects were found [11, 13]. Furthermore, subchronic treatment with EAF promoted an antidepressant effect as well as increased hippocampal cell proliferation in mice [12]. In addition, *T. catigua* treatment provided significant neuroprotection in cerebral ischaemia-reperfusion, and this effect may be attributed to a reduction in reactive oxygen species (ROS) generation [14]. However, the protective mechanism of *T. catigua* against diabetes remains to be further clarified. Thus, considering the broad pharmacological effects attributed to *T. catigua* preparations, especially EAF, we hypothesize that EAF has potentially beneficial effects against type 1 DM, caused by pancreatic β -cell apoptosis, and its related complications such as nephropathy. In this study, we aimed to investigate the anti-diabetic effect of EAF from *T. catigua* and whether this preparation can attenuate the development of diabetic nephropathy in streptozotocin (STZ)-induced type 1 diabetic rats.

Materials and Methods

Plant material and extract preparation

The plant material and crude extract were prepared from the powdered bark of *T. catigua* as previously described [15]. The crude extract was dissolved in water, and EAF was isolated using an ethyl-acetate solution as previously described [13, 16].

The quality of the EAF was evaluated by measuring the amounts of procyanidin B2 (PB2) and epicatechin (EPC) in this fraction against analytical standards by HPLC analysis (Thermo®, San Jose, CA, USA), according to a method developed and validated by our group, as previously described [16]. These standards were chosen for validation because they were available in the appropriate quantities at the laboratory. We solubilized 5 mg of EAF in 3 mL of methanol-water solution (2:8; v/v) and then purified it with a solid-phase extraction cartridge (SPE, SampliQ C18, Agilent Technologies®, Santa Clara, CA, USA) preconditioned with methanol and water. Subsequently, the samples were transferred to a volumetric flask, and the total volume was brought to 10 mL with methanol-water solution (2:8; v/v). The samples were processed in triplicate.

Analysis was performed on an HPLC system with a photodiode array (PDA) spectrophotometric detector module (Model Finnigan Surveyor PDA Plus Detector), integral pumps and degasser (Finnigan Surveyor LC Pump Plus), and an auto sampler equipped with a 10 μ L loop (Finnigan Surveyor Autosampler Plus), and the process was controlled using ChromQuest software (Thermo®, San Jose, CA, USA). The detection wavelength and flow rate were 280 nm and 0.4 mL/min, respectively. We used the Luna PFP(2) column model, 100 Å, 250 x 4.6 mm 5 μ m (Phenomenex®, Torrance, CA, USA), and a guard column (6 x 3 mm, 5 μ m;

Phenomenex®). The mobile phase was eluted by a gradient system that consisted of Phase A, methanol-acetonitrile (75:25; v/v), and Phase B, water, both containing 0.05% trifluoroacetic acid (TFA), as follows: 0 min, 30% phase A; 36 min, 40% phase A; 38 min, 30% phase A, continued at 40 min, followed by a 2 min post-time to re-equilibrate the system.

Ethical approval

The handling of experimental animals was in accordance with Brazilian Law no. 11.794/2008 and approved by The Animal Ethics Committee in Animal Research of the State University of Maringá (protocol number 082/2012).

Animals and experimental design

Adult male Wistar rats, aged 80 days, were housed in the animal facility of the Department of Morphological Sciences in polypropylene cages (45 cm/30 cm/15 cm), 3 animals per cage, under controlled luminosity [12:12 h light-dark cycle (06:00-18:00 h)] and temperature ($22.0 \pm 2^\circ\text{C}$). All animals received standard chow (Nuvilab®, Colombo, Paraná, Brazil) and water *ad libitum*. Food and water intake were evaluated daily, and body weight (BW) weekly.

After one week of adaptation, the animals were divided into four groups ($n = 12$ animals/group): (N) non-diabetic group, (D) type 1 diabetic group, (NC) non-diabetic + EAF group, and (DC) type 1 diabetic + EAF group.

Induction of experimental diabetes

Type 1 DM was induced by a single injection (35 mg/kg BW, i.v.) of STZ (Sigma-Aldrich, St. Louis, MO, USA) dissolved in citrate buffer (0.1 mol/L, pH 4.5) into the penile vein, performed after overnight fasting and under anaesthesia (sodium thiopental; 40 mg/kg BW, i.p.). Diabetes was confirmed 48 h after STZ injection, and the animals that showed fasting blood glucose >350 mg/dL were considered diabetic. Blood samples were obtained from a small cut in the tail vein and measured using a glucometer (ACCU-CHEK® Advantage, Roche Diagnostics, Mannheim, Germany).

EAF treatment

The animals from the NC and DC groups were treated daily with EAF orally administered by gastric gavage (200 mg/kg of BW) for 8 weeks. The EAF was diluted in a drop of glycerin and 0.9% NaCl saline solution (100 mg of EAF in 1 mL of vehicle). The dilution was performed immediately prior to the treatment, and the volume administered was 2 mL/kg of BW. Animals from non-treated groups received vehicle solution without EAF. The treatment occurred between 4:00 and 5:00 p.m.

Euthanasia and sample collection

At the end of the treatment period and after overnight fasting, the animals were anaesthetized with sodium thiopental (i.p. 40 mg/kg BW) for blood sample collection through cardiac puncture. Blood samples were collected into tubes without anticoagulant and centrifuged (3000 rpm for 5 min), and the serum was stored at -80°C for further analyses. Subsequently, the animals were euthanized, and samples of the soleus muscle, retroperitoneal and periepididymal adipose tissue, pancreas and left kidney were removed and weighed.

Biochemical analysis

Blood samples were used for measurement of blood glucose by the glucose oxidase technique (Gold Analisa® Belo Horizonte, Minas Gerais, Brazil) and of creatinine, uric acid, total protein, albumin, globulin, amylase, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) by enzymatic colorimetric methods (Gold Analisa®) according to the manufacturer's instructions.

Histological analysis of the endocrine pancreas

Pancreas samples were fixed in 10% buffered formalin, dehydrated, embedded in histological paraffin and sectioned (5 μm) in non-serial cuts. The tissue sections were deparaffinized, rehydrated and blocked against endogenous peroxidase, washed in 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4) and incu-

bated with 10% non-immune goat serum blocking solution (Histostain-Plus®, Invitrogen, Carlsbad, CA, USA) for 10 min. They were then incubated with monoclonal primary antibodies against insulin (1:500, Sigma®, St. Louis, MO, USA) or against PCNA (1:100, Zymed®, San Francisco, CA, USA) for 60 min. After being washed (0.01 mol/L PBS), sections were incubated with a biotinylated secondary antibody (Histostain-Plus®) for 10 min, washed and incubated with diaminobenzidine chromogenic solution (Histostain-Plus®) for 15 min, washed again, and finally counterstained with haematoxylin. The morphometric analyses were performed using digital images (TIFF 24-bit colour, 2560 × 1920 pixels) obtained with a light microscope (Olympus BX41, Tokyo, Japan) and a camera QColor 3 Olympus. Quantitative analyses of the islets number were performed using digital images (×20 magnification) of 6 different fields from each animal (n = 6 animals/group). Analyses of islet area, islet insulin immunodensity and PCNA-positive cells were performed using 40 digital images (×400 magnification) from each animal (n = 6 animals/group). These analyses were performed using Image-Pro Plus 4.5 software (Media Cybernetics, Silver Spring, MD, USA).

Histological analysis of the kidney

Kidney samples were fixed in 10% buffered formalin, dehydrated and embedded in histological paraffin. Kidney tissues were sectioned (5 µm) in non-serial cuts and stained with Heidenhain's Azan trichrome. The morphometric analyses were performed by measuring the positive collagen area in relation to the total area of digital images from the glomerular region of the kidney, using 40 digital images (×200 magnification) from each animal (n = 6 animals/group). These analyses were performed using Image-Pro Plus 4.5 software.

Statistical analysis

Data were expressed as the means ± standard error (SEM). Significant differences between mean values of different groups were determined by one-way analysis of variance (ANOVA) with post hoc Tukey's tests. Differences were considered significant at $p < 0.05$. Statistical analysis and graphs confection were performed using GraphPad Prism version 6.0 software for Windows (GraphPad Software Inc., San Diego, CA, USA).

Results

Chromatographic analysis of EAF

The chromatogram of EAF is shown in Fig. 1. The concentrations of PB2 and EPC in the EAF samples were 37.2 ± 0.84 and 20.3 ± 0.74 µg/mL, respectively. On the other hand, in the EAF analytical solution, the PB2 and EPC concentrations were 74.4 and 40.6 mg/g, respectively.

Effect of EAF from *T. catigua* on biometric parameters

The STZ-diabetic rats showed low body weight throughout the experimental period compared to non-diabetic rats (Fig. 2A). This result is illustrated by their decrease in body weight gain (178%; Fig. 2B). EAF treatment attenuated the body weight loss from the fifth to 8th weeks of treatment in the DC group.

As shown in Fig. 2C and 2D, the retroperitoneal and periepididymal fat were decreased by approximately 94% in the STZ-diabetic group compared to non-diabetic rats. On the oth-

Fig. 1. HPLC analyses of EAF from *T. catigua*. Procyanidin B2 (PB2; 1), mixture of catechin and chlorogenic acid (2), cinchonain IIb (3), cinchonain IIa (4), epicatechin (EPC; 5), mixture of cinchonains Ia and Ib (6). Flow rate 0.4 mL/min, detection wavelength 280 nm.

Fig. 2. Effect of EAF from *T. catigua* on biometric parameters. Body weight throughout experimental period (A), body weight gain (B), retroperitoneal adipose tissue (C), periepididymal adipose tissue (D) and soleus muscle (E) weight. Data are presented as the mean $\pm$ SEM. One-way ANOVA and post hoc Tukey's test. N: non-diabetic group, D: type 1 diabetic group, NC: non-diabetic + EAF group and DC: type 1 diabetic + EAF group.

er hand, in the DC group, this fat weight loss was inhibited in both adipose tissue depots by EAF. The STZ-diabetic group had a reduction of 39% in soleus muscle mass. However, EAF treatment inhibited this parameter by 25% in the DC group (Fig. 2E).

Effect of EAF from *T. catigua* on food and water intake

Figure 3 illustrate the effect of EAF on food and water intake. The non-diabetic groups (N and NC groups) show similar curves of food and water intake (Fig. 3A and 3C, respectively). STZ-diabetic rats show higher food and water intake throughout the experimental period compared to the control rats. The area under the curve (AUC) values of food and water intake increased by 64% and 361%, respectively, in the STZ-diabetic group compared to the non-diabetic group (Fig. 3B and 3D, respectively). Interestingly, treatment with EAF attenuated the increase in food and water intake, reducing it by approximately 23% and 42%, respectively, in the DC group.

Effect of EAF from *T. catigua* on biochemical parameters

Table 1 shows the effect of EAF on biochemical parameters. Blood glucose concentration was higher in STZ-diabetic rats compared to non-diabetic rats (403%; $p < 0.05$). However, EAF treatment reduced significantly (24%) blood glucose in DC group ($p < 0.05$).

STZ-diabetic rats exhibited low serum creatinine levels, and this parameter did not change in STZ-diabetic rats treated with EAF. On the other hand, serum levels of uric acid and amylase increased significantly in STZ-diabetic rats compared to non-diabetic rats ($p < 0.05$). Nevertheless, EAF treatment improved these parameters in the DC group.

STZ-diabetic rats showed significant decreases in serum levels of total protein (19%), albumin (13%) and globulin (28%). Oral treatment with EAF reversed these parameters in the DC group. Furthermore, ALP, AST, ALT and GGT were significantly increased in the serum of STZ-diabetic rats compared to control rats ($p < 0.05$). EAF treatment promoted a marked reduction in these enzymes in the DC group ($p < 0.05$).

Fig. 3. Effect of EAF from *T. catigua* on food and water intake. Food intake throughout experimental period (A), AUC of food intake (B), water intake throughout experimental period (C) and AUC of water intake (B). Data are presented as the mean $\pm$ SEM. One-way ANOVA and post hoc Tukey's test. N: non-diabetic group, D: type 1 diabetic group, NC: non-diabetic + EAF group and DC: type 1 diabetic + EAF group.

Table 1. Effect of EAF from *T. catigua* on biochemical parameters. Data are presented as the means $\pm$ SEM. *values differ from non-diabetic group ($p < 0.05$), #values differ from diabetic group ($p < 0.05$) and ns, not significantly different. One-way ANOVA and post hoc Tukey's test. N: non-diabetic group, D: type 1 diabetic group, NC: non-diabetic + EAF group and DC: type 1 diabetic + EAF group

	N	D	NC	DC
Blood glucose (mg/dL)	109.0 $\pm$ 1.24	547.9 $\pm$ 13.21*	105.3 $\pm$ 5.33	414.6 $\pm$ 34.87#
Creatinine (mg/dL)	0.36 $\pm$ 0.05	0.20 $\pm$ 0.02*	0.36 $\pm$ 0.02	0.25 $\pm$ 0.02 ^{ns}
Uric acid (mg/dL)	50.86 $\pm$ 2.82	90.33 $\pm$ 2.72*	48.00 $\pm$ 1.53	69.67 $\pm$ 3.65#
Total protein (g/dL)	5.71 $\pm$ 0.05	4.61 $\pm$ 0.05*	5.76 $\pm$ 0.10	5.17 $\pm$ 0.06#
Albumin (g/dL)	2.81 $\pm$ 0.03	2.45 $\pm$ 0.03*	2.80 $\pm$ 0.11	2.76 $\pm$ 0.08#
Globulin (g/dL)	2.91 $\pm$ 0.03	2.10 $\pm$ 0.04*	2.93 $\pm$ 0.04	2.40 $\pm$ 0.04#
Amylase (U/dL)	91.6 $\pm$ 2.16	175.4 $\pm$ 5.52*	93.1 $\pm$ 1.64	131.2 $\pm$ 2.47#
ALP (U/L)	138.6 $\pm$ 7.69	1078.3 $\pm$ 15.86*	152.7 $\pm$ 7.95	759.4 $\pm$ 26.23#
ALT (U/L)	224.7 $\pm$ 22.47	658.3 $\pm$ 65.85*	222.1 $\pm$ 25.87	419.1 $\pm$ 67.10#
AST (U/L)	494.0 $\pm$ 44.83	693.6 $\pm$ 22.73*	366.4 $\pm$ 61.96	335.9 $\pm$ 15.60#
GGT (U/L)	11.57 $\pm$ 1.0	44.00 $\pm$ 4.6*	12.29 $\pm$ 1.5	18.71 $\pm$ 4.5#

Effect of EAF from *T. catigua* on the morphology of the endocrine pancreas

Histopathological examination of the endocrine pancreas revealed a large reduction in the number of pancreatic β -cells and in the size and number of pancreatic islets in the STZ-diabetic group compared to the non-diabetic group (Fig. 4A–4H). Quantitative morphometric analysis reveals significant reductions in the number, area and insulin immunodensity of pancreatic islets. (Fig. 4I, 4J and 4K). Moreover, the pancreatic islets from the STZ-diabetic group show uneven distribution of pancreatic β -cells, as shown in Fig. 4F. Interestingly, EAF treatment significantly improved the number, area and insulin immunodensity of pancreatic islets in the DC group and did not change these parameters in the NC group (Fig. 4I, 4J and 4K). Figure 5 shows that EAF induced pancreatic β -cell proliferation (PCNA-positive cells) in both treated groups.

Fig. 4. Effect of EAF from *T. catigua* on morphology of endocrine pancreas. Representative photomicrographs (A–D, $\times 20$ magnification, scale bars = 1000 μm and E–H, $\times 400$ magnification, scale bars = 50 μm) show pancreatic sections immunostained with anti-insulin antibody. Non-diabetic group (A and E), diabetic group (B and F), non-diabetic + EAF group (C and G) and diabetic + EAF group (D and H). Quantitative analysis is shown in (I), (J) and (K). Data are presented as the mean $\pm$ SEM. One-way ANOVA and post hoc Tukey's test. N: non-diabetic group, D: type 1 diabetic group, NC: non-diabetic + EAF group and DC: type 1 diabetic + EAF group.

Fig. 5. Effect of EAF from *T. catigua* on cellular proliferation of endocrine pancreas. Representative photomicrographs (A–D, $\times 400$ magnification, scale bars = 50 μm) show pancreatic sections immunostained with anti-PCNA antibody. Non-diabetic group (A), diabetic group (B), non-diabetic + EAF group (C) and diabetic + EAF group (D). Images show nuclei immunolabelled for PCNA in the pancreatic islets of the *T. catigua*-treated groups (arrows). Quantitative analysis of the PCNA-positive cells (E). Data are presented as the mean $\pm$ SEM. One-way ANOVA and post hoc Tukey's test. N: non-diabetic group, D: type 1 diabetic group, NC: non-diabetic + EAF group and DC: type 1 diabetic + EAF group.

Effect of EAF from *T. catigua* on the morphology of the kidney

As shown in Fig. 6E and 6F, both the absolute and relative weight of kidneys from STZ-diabetic rats were significantly increased (21% and 96%, respectively) compared to non-diabetic rats. In agreement with these data, as observed in Fig. 6B and 6G, the renal histopathological examination demonstrates a greater area of collagen in STZ-diabetic rats than in the control ones. Interestingly, these parameters were restored to normal by the EAF treatment.

Fig. 6. Effect of EAF from *T. catigua* on morphology of kidney. Representative photomicrographs (A–D, $\times 200$ magnification, scale bars = 100 μ m) show renal sections stained with Heidenhain's Azan trichrome. Non-diabetic group (A), diabetic group (B), non-diabetic + EAF group (C) and diabetic + EAF group (D). Kidney mass (E), relative kidney mass (F) and quantitative analysis of the collagen area (G). Data are presented as the mean $\pm$ SEM. One-way ANOVA and post hoc Tukey's test. N: non-diabetic group, D: type 1 diabetic group, NC: non-diabetic + EAF group and DC: type 1 diabetic + EAF group.

Discussion

The present study confirms the hypothesis that EAF treatment has an anti-diabetic effect. Given that, for the first time, this work shows that STZ-diabetic rats treated with EAF presented a significant attenuation in body weight loss as well as in the higher ingestion of food and water that features prominently in diabetes. Other studies observed that treatment with natural products improved these parameters [17, 18]. Additionally, reduction of the blood glucose levels and an improvement in several others diabetic hallmark parameters were found herein. Furthermore, anti-insulin immunohistochemical analysis shows significant increased pancreatic β -cell number and islet size in the DC group as a result of β -cell proliferation, ascertained through labelling by PCNA immunohistochemistry. In addition, our histopathological studies showed that EAF promoted reduction in the kidney tissue damage that is caused by diabetes.

A striking feature of STZ-type 1 diabetic rats is a severe reduction of body weight caused by loss of adipose and muscular tissue; these characteristics are known as cachexia [19]. Adipose and muscular tissue need insulin signalling for glucose uptake; without insulin, these tissues show a significant reduction of glucose uptake that compromises their overall metabolism [2, 17].

The main therapeutic goal of diabetes treatment is to control blood glucose levels [4, 17]. Here, we show the effectiveness of the use of *T. catigua* as a powerful anti-diabetic agent, demonstrating improvements in the hyperglycaemia, polydipsia, and polyphagia observed in STZ-diabetic rats. In light of these results, new findings are needed for better understanding the hypoglycaemic effect of *T. catigua*. On the other hand, recent studies have shown anti-inflammatory and antioxidant effects of PB2 and EPC, which exert positive effects by improving diabetes comorbidities. They were observed to bring improvement in insulin sensitivity and to exert a protective effect against diabetic nephropathy and cardiomyopathy [20, 21].

In this study, diabetic animals exhibited low blood creatinine as well as decreased total protein, albumin and globulin levels compared to control animals. Interestingly, EAF treatment restored total protein, albumin and globulin concentrations nearly to control group values. No significant effect was observed in blood creatinine in the DC group. These results

are consistent with improvement of diabetes and kidney function as observed in other studies [18, 22]. Furthermore, previous studies have demonstrated lower serum creatinine levels and increased serum uric acid in STZ-diabetic animals and in type 2 diabetic humans [23]. Similarly, in this work, serum creatinine was lower and uric acid higher in the STZ-diabetic group, which could suggest renal impairments associated with diabetic complications [22]. Moreover, serum amylase was significantly elevated in STZ-diabetic rats, which is compatible with diabetic ketoacidosis [24]. However, we found that EAF attenuated diabetes-induced elevations in serum uric acid and amylase. Phenolic compounds present in *T. catigua* are probably, at least in part, the main agents responsible for the protective effect of EAF on kidney function as indicated by our results. In addition, previous studies have demonstrated that natural products exert protective effects on kidney function by mediating many of those parameters [18, 22, 25].

The increases in the activity levels of serum ALP, ALT and AST enzymes have been associated with liver damage [26]. In the present study, a significant rise in the ALP, ALT, AST and GGT serum levels was observed in STZ-induced diabetic rats, whereas the diabetic group treated with EAF showed a significant improvement in these parameters. Several studies have shown that natural products from plants exert beneficial effects by reducing the high levels of ALP, ALT and AST in diabetic individuals, which leads an improvement in hepatic dysfunction [22, 26-28].

STZ-induced type 1 diabetes has been described as a useful experimental model for the study of diabetes because of the selective pancreatic β -cell destruction caused by this drug [29]. Here, we show a significant reduction in the number, area, and insulin immunodensity of pancreatic islets in STZ-diabetic rats that is compatible with pancreatic β -cell death caused by STZ. In particular, STZ induces the accumulation of superoxide, hydroxyl radicals and nitric oxide, which are known to be cytotoxic. Increased ROS production by STZ cause pore formation in mitochondria, resulting in disruption of the electrical potential of mitochondrial membrane, unbalancing pancreatic β -cell metabolism and insulin release [30, 31].

Interestingly, our hypothesis that EAF has anti-diabetic effects was confirmed by the findings shown in this study. Here, we depict the effectiveness of EAF treatment against diabetic complications; also, we suggest that the effects of EAF treatment in ameliorating diabetic injuries in STZ-diabetic rats may be closely associated with the increase of pancreatic β -cell proliferation as observed here, which partially restores the normal morphology and endocrine function of the damaged pancreatic islets caused by STZ in the rats. These findings indicate that EAF may be useful as a potential pharmacological agent to protect against pancreatic β -cell damage caused by oxidative stress associated with diabetes. However, the protective mechanism of EAF on pancreatic islets remains to be further clarified. We hypothesized that EAF has potentially beneficial effects against pancreatic β -cell apoptosis that may derive from its anti-inflammatory capacity and inhibitory actions against free radicals [13].

During the course of diabetes, hyperglycaemia induces AGE formation, pro-inflammatory cytokine overexpression and oxidative stress, promoting kidney damage [27, 32]. In the present study, STZ-diabetic rats show a large area stained by Azan trichrome, indicating ECM expansion and collagen deposition. This parameter is compatible with renal fibrosis. Thus, these results were compatible with the kidney function parameters such as low creatinine and serum protein and high uric acid found in this work. On the other hand, EAF treatment inhibited ECM expansion and restored renal morphology in the DC group to a normal pattern. Obviously, EAF treatment may have improved morphology and renal function due to reduction of blood glucose. However, beneficial effects on renal function promoted by plant compound treatment, likely because of beneficial effects of polyphenols on free radicals and inflammatory status, have been observed [18, 25]. The present study showed that EAF from *T. catigua* improves glucose homeostasis and endocrine pancreas function and inhibits the development of diabetic nephropathy in STZ-induced type 1 diabetic rats. All together, these observations suggest that *T. catigua* could be useful as an anti-diabetic agent.

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Disclosure Statement

The authors have no competing interests.

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