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Hypoglycemic, hypolipidemic and antioxidant potential of *Myrcia pubipetala* in an animal model of type 1 diabetes

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ABSTRACT. *Myrcia* genus plants, like *Myrcia pubipetala*, traditionally used as hypoglycemic agents, hold promise for Type 1 Diabetes Mellitus (DM1) research but need more comprehensive chemical and pharmacological investigation. This is an experimental study involving controlled administration of treatments to a group of rats to assess their effects compared with a control group, investigating the effects of *M. pubipetala* on alloxan-induced Type 1 Diabetes Mellitus in rats. After the induction of diabetes, the rats received the hydroalcoholic extract (HAE) or aqueous fraction (AF) (25, 50, 100, or 150 mg kg^{-1}) of *M. pubipetala*, or water. The results showed that diabetic rats presented cell damage in kidneys, oxidative stress, and high levels of glucose and triglycerides in their plasma and erythrocytes. The HAE (150 mg kg^{-1}), *per se*, reduced lipid and protein oxidation, and the AF (150 mg kg^{-1}) decreased lipoperoxidation. AF (150 mg kg^{-1}), per se, decreased triglyceride levels. Conclusion: treatment with HAE and AF reduced oxidative damage, positively modulated antioxidant defenses, and exerted hypoglycemic and hypolipidemic effects, representing a potential adjuvant treatment for diabetes.

Keywords: diabetes mellitus; Myrtaceae; oxidative stress.

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Introduction

With the current urbanization, the epidemiological transition, and the increase in the prevalence of sedentary lifestyles, among other factors, diabetes mellitus (DM) is a growing and alarming problem due to its significant impact on those affected by the disease. According to the International Diabetes Federation (IDF, 2021), the total number of the world's population with diabetes is predicted to rise to 643 million (11.3%) by 2045. Almost half of the currently available medications have been developed through bioprospecting. The available treatments for diabetes are no different. Of the bioactive chemical compounds used to treat DM, 9% are synthetic substances, while 23% are natural products, and 64.10% are synthetic but were inspired by a natural pharmacophore (Newman & Cragg, 2020).

Some species of the *Myrcia* genus are commonly referred to as 'vegetable insulin', and have been employed in traditional medicine for diabetes treatment (Basting et al., 2014). This group includes plants such as *Myrcia* (*M.*) *punicifolia* (Kunth) DC., *M. speciosa* (Amsh.) Mc Vaugh, *M. amazonica* DC., *M. citrifolia* (Aubl.) Urb., *M. guianensis* (Aubl.) DC., and *M. uniflora* DC. (Silva, Rosario, Secco, & Zoghbi, 2015). This endemic Brazilian species is distributed from Bahia to Rio Grande do Sul, yet there exists a scarcity of literature addressing its chemical or pharmacological composition.

The search for new treatments for DM that target glycemic control and possess antioxidant potential is intriguing because DM represents a group of diseases with the main characteristic of high blood glucose levels due to a disturbance in the secretion or action of the insulin hormone, altering the metabolism of lipids, carbohydrates, and proteins (Cotran, Kumar, & Collins, 2000). Persistent exposure to hyperglycemia causes endothelial damage, incurring, amongst other alterations, increased activity of the polyol pathway, non-enzymatic glycation of proteins

and oxidative stress, with an increase in free radicals (FR) and changes in protein kinase C (Schalkwijk & Stehouwer, 2005) in the body. FR and reactive oxygen species (ROS) are constantly produced in the body in metabolic pathways that, when in excess, lead to oxidative stress, causing damage to essential organic structures. The body's antioxidant system, which is made up of endogenous enzymes and exogenous molecules acquired in food, can neutralize such reactive structures and, thus, combat cellular changes of oxidative stress. On certain occasions, as in DM, when the levels of antioxidants are insufficient to protect the organism, FR and ROS prevail and can cause diseases (Rains & Jain, 2011; Novoselova et al., 2021).

T1DM is an autoimmune polygenic disease developed from the destruction of β cells, causing absolute insulin deficiency (American Diabetes Association [ADA], 2010). If inadequately treated, it can cause irreversible chronic complications, such as dysfunction and failure of organs, such as kidneys, eyes, nerves, heart, and blood vessels, leading to complications such as neuropathy, nephropathy, retinopathy, myocardial infarction, vascular accidents, and infections (Forbes & Cooper, 2013). Thus, alternative strategies for the treatment are necessary for better management of the pathology, to improve quality of life, and to minimize the side effects of the disease. Added to this are the high costs of modern medicines (*Sociedade Brasileira de Diabetes* [SBD], 2019).

Due to various mechanisms of action, extracts from plants of the Myrtaceae family, such as *Eucalyptus globulus*, *Eugenia jambolana*, *Syzygium alternifolium* have shown hypoglycemic effects and protection against the disease (Mauro, 2015). Within Myrtaceae, *Myrcia* constitutes one of the largest genera with wide distribution in several Brazilian floristic domains like the Atlantic Forest, the Amazon Forest, and Cerrado (Fontana, Gasper, & Sevegnani, 2014). Considering the hypoglycemic and antioxidant potential of the Myrtaceae family, the present study evaluated the possible hypoglycemic, lipid-lowering, and antioxidant activities of the crude hydroalcoholic extract (HAE) and the aqueous fraction (AF) obtained from the leaves of *Myrcia pubipetala,* in an animal model of T1DM.

Materials and methods

Preparation of extracts from the leaves of *Myrcia pubipetala*

Leaves of *Myrcia pubipetala* were collected in Blumenau, Santa Catarina State, Brazil (26º 90' 63" S, 49º 08' 01" W) in March 2020. The identification of the plant species was made by the botanist André Luís de Gasper. Plant material was deposited at the Herbarium FURB under registration number 34168. *Myrcia pubipetala* leaves were dried in an air circulation oven and then ground in a knife mill. Leaves were then subjected to extraction by maceration with a 70% hydroalcoholic solution for seven days. The extract was filtered using a Buchner funnel coupled with a vacuum pump and concentrated in a rotary evaporator under reduced pressure at a temperature below 60 ºC to obtain the hydroalcoholic crude extract (HAE). The crude extract was resuspended in a 20% hydroalcoholic solution and partitioned into solvents, yielding the aqueous fraction (AF).

Animals

Wistar rats (*Rattus norvegicus*, males, 300 g, 21 days) were used under the approval of the local Ethics Committee of the *Universidade Regional de Blumenau* (protocol 11/2020). The conditions of environment, lighting, accommodation, and nutrition followed the recommendations required by the Guide for the Care and Use of Laboratory Animals, 1996.

Experimental design

For the induction of T1DM, Aloxan (2%) (150 mg kg^{-1}) was administered once, intraperitoneally, to animals after a 24 hours fast. Six hours later, 10% glucose was provided as the only water source for one day. One day after the administration of Aloxan, the animals with blood glucose above 200 mg dL^{-1} were selected for the experiment, and those with insufficient values were reinduced.

Eighty rats were divided into ten groups (n = 8) as follows: Control group: received water intraperitoneal (i.p) injection (once) and water via oral gavage once a day for 15 days; T1DM group: received an i.p. alloxan injection at a dose of 150 mg kg^{-1} and water via oral gavage once a day for 15 days; Control groups extracts: received water by i.p. (1 time) and HAE or AF of *Myrcia pubipetala* via oral gavage (25, 50, 100 or 150 mg kg^{-1}) once daily for 15 days and T1DM Groups + extracts: received an i.p. injection of alloxan at a dose of 150 mg kg^{-1} and HAE or AF via oral gavage (25, 50, 100 or 150 mg kg^{-1}) once a day for 15 days. Treatment started after

confirmation of diabetes. Twelve hours after the last treatment, the animals were sacrificed by decapitation in the absence of anesthesia, and then whole blood was collected and the kidneys removed for biochemical tests. The doses of HAE and AF (25, 50, 100, and 150 mg kg^{-1}) were chosen based on previous studies (Ravi, Ramachandran, & Subramanian, 2004; Ravi, Rajasekaran, & Subramanian, 2005; Kar, Choudhary, & Bandyopadhyay, 2013).

Biochemical studies

'Erythrocyte and plasma preparation' - The whole blood was collected in anticoagulant, centrifuged at 1000 rpm for 10 min, and the plasma was separated and refrigerated. Erythrocytes were washed three times with cold saline solution (0.153 mol L^{-1} sodium chloride) and lysates were prepared by the addition of 1 mL of distilled water to 100 µL washed erythrocytes and maintained frozen at −80 °C until determination of the antioxidant enzyme activities. For antioxidant enzyme activity determination, erythrocytes were frozen and thawed three times, and centrifuged at 13,500 × g for 10 min. The supernatant was diluted in order to contain approximately 0.5 mg mL^{-1} of protein.

'Tissue preparation' - Kidneys were removed, unencapsulated, kept on ice-cold buffered sodium phosphate (20 mM, pH 7.4, 140 mM KCl) and homogenized. Homogenates were prepared using a Potter Elvehjem homogenizer (Remi motors, Mumbai, India) by passing 5 pulses and centrifuged at 800 × g for 10 min. at 4 °C before discarding nuclei and cell debris. The pellet was discarded, and the supernatant was saved in aliquots and stored at −80 °C for assaying the activity of antioxidant enzymes, damage to proteins and estimation of lipid peroxidation (Lima et al., 2018).

'Glucose and Triglycerides dosage' - Glucose and triglyceride levels were measured using glucose and triglyceride kits (reference 133 and 87 from Labtest, respectively), following the manufacturer's manual.

'Thiobarbituric acid reactive substances (TBA-RS)' - TBA-RS were determined according to the consolidated method described by Ohkawa, Ohishi, and Yagi (1979) that measures malondialdehyde (MDA), a product of lipoperoxidation, produced mainly by hydroxyl free radicals. Initially, plasma and kidney in 1.15% KCl was mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 60 min. TBA-RS were determined by the absorbance at 535 nm. A calibration curve was obtained using 1,1,3,3-tetramethoxypropane as the MDA precursor and each curve point was subjected to the same treatment as that of the supernatants. TBA-RS content was expressed as nanomoles of MDA formed per milligram of protein.

'Total sulfhydryl content' - Total sulfhydryl content was determined according to the method of Aksenov and Markesbery (2001), and is based on the reduction of dithionitrobenzoic acid (DTNB) by thiols, generating a yellow derivative (TNB), which is measured spectrophotometrically at 412 nm. For the assay, 50 µL of homogenate was added to 1 mL of phosphate buffered saline (PBS), pH 7.4, containing 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction was started by the addition of 30 µL of 10 mM DTNB and incubated for 30 min. at room temperature in the dark. Analysis of a blank (DTNB absorbance) was also performed. The results were expressed as nmol TNB mg^{-1} protein.

'Protein carbonyl content' - Protein carbonyl content and measured was assayed by Reznick and Packer (1994), based on the reaction of protein carbonyls with dinitrophenylhydrazine to form dinitrophenylhydrazone, a yellow compound that is measured spectrophotometrically at 370 nm. Briefly, 200 µL of homogenate were added to plastic tubes containing 400 µL of 10 mM dinitrophenylhydrazine (prepared in 2M HCl). Samples were kept in the dark for 1h and vortexed every 15 min. Subsequently, 500 µL of 20% trichloroacetic acid were added to each tube. The mixture was vortexed and centrifuged at 14,000 rpm for 3 min. and the supernatant obtained was discarded. The pellet was washed with 1 mL ethanol/ethyl acetate (1:1 v v^{-1}), vortexed and centrifuged at 14,000 rpm for 3 min. The supernatant was discarded and the pellet resuspended in 600 µL of 6M guanidine (prepared in a 20 mM potassium phosphate solution, pH 2.3), before vortexing and incubating at 60 ºC for 15 min. Samples were then centrifuged at 14,000 rpm for 3 min. and the supernatant was used to measure absorbance at 370 nm (UV) in a quartz cuvette. Results were reported as carbonyl content (nmol mg^{-1} protein).

'Catalase Assay (CAT)' - CAT activity was determined by the consolidated method of Aebi (1984). The method is based on the disappearance of hydrogen peroxide (H_2O_2) at 240 nm in a reaction medium containing 25 µL of the sample and 600 µL of 10 mM potassium phosphate buffer, pH 7.0, 20 mM H_2O_2. One CAT unit is defined as 1 µmol of H_2O_2 consumed per minute and the specific activity is calculated as CAT units mg^{-1} protein.

'Glutathione Peroxidase Assay (GSH-Px)' - The activity of GSH-Px was measured by the method of Wendel (1981), using tert-butyl-hydroperoxide as substrate. The decomposition of NADPH was monitored in a spectrophotometer at 340 nm for 4 min. The medium contained 2.0 mM GSH, 0.15 U mL^{-1} GSH reductase, 0.4

mM azide, 0.5 mM tert-butyl-hydroperoxide and 0.1 mM NADPH. One GSHPx unit is defined as 1 µmol of NADPH consumed per minute and the specific activity is reported as GSH-Px units mg^{-1} of protein.

'Superoxide Dismutase Assay (SOD)' - SOD activity was analyzed using consolidated methodology, as described by Marklund (1985). This procedure is highly dependent on superoxide (O_2•-), which is a substrate for SOD. 15 µL of each sample was added to 215 µL of a mixture containing 50 µM Tris buffer, 1 µM EDTA, pH 8.2, and 30 µM CAT. Subsequently, 20 µL of pyrogallol was added and the absorbance was measured every 30 seconds for 3 min. at 420 nm using a spectrophotometer. Inhibition of autoxidation of pyrogallol occurs in the presence of SOD, the activity of which can be indirectly tested spectrophotometrically. One unit of SOD is defined as the amount of SOD required to inhibit 50% of the auto-oxidation of pyrogallol and the specific activity is reported as SOD units mg^{-1} protein.

'Protein determination' - Protein was measured by the method of Lowry, Rosebrough, Farr, and Randall (1951), using serum bovine albumin as standard.

'Statistical analysis' - The Kolmogorov-Smirnov normality test was performed to confirm a parametric distribution. The results of the different groups were represented as mean ± standard deviation, analyzed using the SPSS program for Windows, version 12 (SPSS, Chicago, IL, USA), using analysis of variance (ANOVA), followed by the posthoc test Duncan's test for comparison between the means of the groups, and p values < 0.05 were considered significant.

Results and discussion

The present study evaluated the effects of the chronic administration of HAE and AF, obtained from *Myrcia pubipetala* leaves, on the alterations caused by alloxan-induced T1DM (namely, hyperglycemia, hypertriglyceridemia and parameters of oxidative stress) in the blood and kidneys of rats. In DM, the homeostasis of the antioxidant defense system is interrupted, and persistent hyperglycemia causes an increase in the mitochondrial production of ROS, establishing oxidative stress. In this scenario, macromolecules suffer damage to their structures, thus contributing to the progression of diabetic complications and, consequently, generating damage in different tissues (Schmidt, 2018).

CAT metabolizes H_2O_2, while GSH-Px removes H_2O_2 and other organic hydroperoxides, and SOD catalyzes the dismutation of O_2^- into O_2 and H_2O_2 (Halliwell & Gutteridge, 2015). Measurement of TBA-RS evaluates lipid oxidation and reflects membrane peroxidation (Halliwell & Whiteman, 2004). Protein carbonyl derivatives can be generated through the oxidative cleavage of proteins via the α-amidation pathway or by the oxidation of glutamyl side chains (Berlett & Stadtman, 1997). The total sulfhydryl content is a parameter that measures the sulfhydryl content of the sample, which is inversely correlated with oxidative damage to the protein (Berlett & Stadtman, 1997).

Hyperglycemia (Figure 1a) and hypertriglyceridemia (Figure 1b) was observed in rat plasma, and HAE (50, 100, and 150 mg kg^{-1}) ($F_{9.43} = 610.649$ $p < 0.001$) partially reversed hyperglycemia and partially reversed hypertriglyceridemia (50 mg kg^{-1}) and wholly reversed hypertriglyceridemia (100 and 150 mg kg^{-1}) ($F_{9.46} = 33.623$; $p < 0.001$).

In erythrocytes from diabetes-induced rats, there were elevated levels of TBA-RS (Figure 2a) and reduced activities of CAT (Figure 2d), SOD (Figure 2e), and GSH-Px (Figure 2e), when compared to the control groups. There was no change in the total contents of sulfhydryls (Figure 2b) ($F_{5.30} = 0.506$; $p > 0.05$) and carbonyls (Figure 2c) ($F_{5.30} = 0.422$; $p > 0.05$). After the administration of 100 and 150 mg kg^{-1} HAE, there was a total reversal of the alterations in TBA-RS ($F_{9.49} = 83.585$; $p < 0.001$), CAT ($F_{9.48} = 56.150$; $p < 0.001$), SOD ($F_{9.47} = 19.440$; $p < 0.001$) and GSH-Px ($F_{9.48} = 22.796$; $p < 0.001$).

For the kidney evaluation, there was an increase in the levels of TBA-RS (Figure 3a), the content of carbonylated proteins (Figure 3c), and the activity of the antioxidant enzymes, SOD (Figure 3e) and GSH-Px (Figure 3f), in rats with T1DM, when compared to the control groups. On the other hand, there was no change in the total sulfhydryl content (Figure 3b) nor the CAT activity (Figure 3d) ($F_{5.30} = 0.803$; $p > 0.05$). Additionally, HAE (150 mg kg^{-1}) treatment reduced TBA-RS levels, increased total sulfhydryl content ($F_{5.30} = 10.817$; $p < 0.001$), and reduced carbonylated protein content when compared with control and T1DM groups. HAE reversed the increase in TBA-RS (Figure 3a) levels (150 mg kg^{-1}) ($F_{9.49} = 50.694$; $p < 0.001$) and in the total content of carbonyl proteins (Figure 3c) (100 and 150 mg kg^{-1}) ($F_{9.38} = 62.400$; $p < 0.001$), as well as the increased activities of SOD (Figure 3e) ($F_{9.49} = 34.462$; $p < 0.001$) and GSH-Px (Figure 3f) (150 mg kg^{-1}) ($F_{9,48} = 28.680$; $p < 0.001$) in the kidneys of diabetic rats.

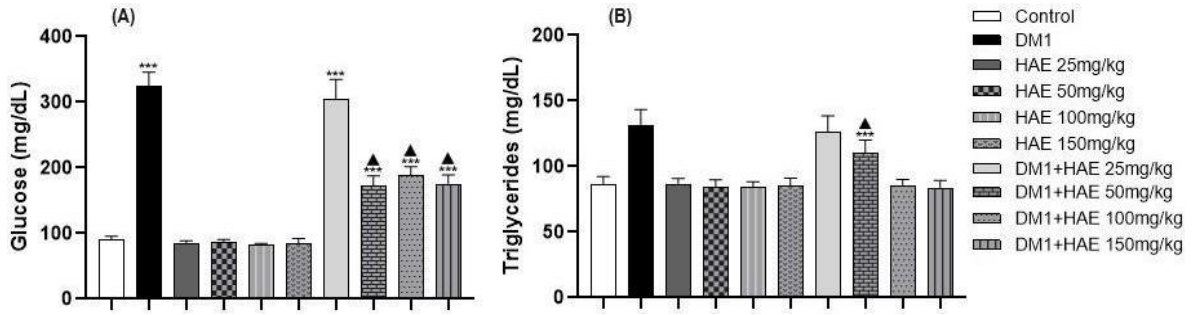

Figure 1. Effects of the chronic administration of HAE of *Myrcia pubipetala,* in the presence or absence of alloxan-induced diabetes, on plasma levels of glucose (A) and triglycerides (B). Glucose and triglycerides are expressed in mg dL^{-1}. Data correspond to the mean ± SD for 6 independent experiments (animals), carried out in duplicate. Unlike the control, ***p < 0.001; different from the diabetic group, ▲ p < 0.001 (Multiple Duncan Test). HAE: hydroalcoholic crude extract; T1DM: Type 1 Diabetes Mellitus.

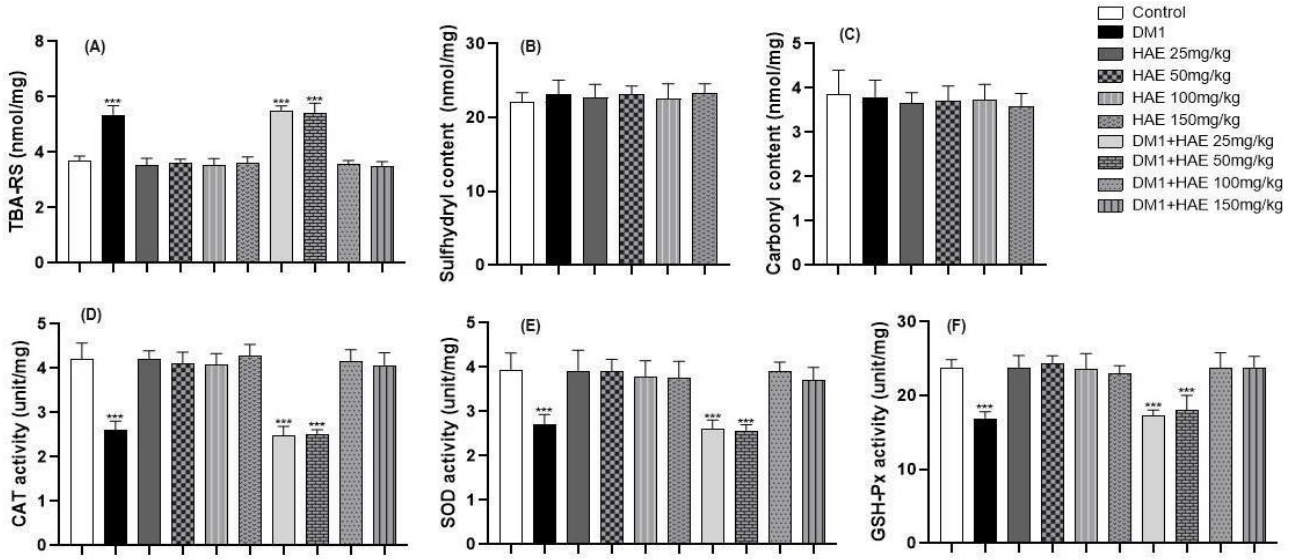

Figure 2. Effects of the chronic administration of the hydroalcoholic crude extract (HAE) of *Myrcia pubipetala* (Ep), in the presence or absence of alloxan-induced diabetes, on thiobarbituric acid reactive substances (TBA-RS) (A), total sulfhydryl content (B), total carbonyl protein content (C), catalase (CAT) activity (D), superoxide dismutase (SOD) (E) and glutathione peroxidase (GSH-Px) (F) in rat blood. TBA-RS is expressed as a nmol of malondialdehyde per mg of protein. The total sulfhydryl content is expressed as nmol of TNB per mg of protein. The total carbonyl protein content is expressed in nmol of carbonyl per mg of protein. Enzyme activity is expressed in units per mg of protein. Data correspond to mean ± SD for 6-7 independent experiments (animals) performed in duplicate. Unlike the control, ***p < 0.001 (Duncan's Multiple Test). HAE: hydroalcoholic crude extract; T1DM: Type 1 Diabetes Mellitus.

Subsequently, the effects of the chronic administration of the AF, obtained from *Myrcia pubipetala* leaves, on the changes caused by alloxan-induced T1DM were evaluated for the same parameters in plasma and kidney. The administration of the AF from *Myrcia pubipetala* caused a partial reversal of T1DM effects on glucose levels (Figure 4a) at doses of 50, 100, and 150 mg kg^{-1} ($F_{9.44}$ = 417.293; p < 0.001) and a total reversal of the increase in triglycerides (Figure 4b) at doses of 50 and 100 mg kg^{-1} ($F_{9.50}$ = 117.180; p < 0.001) in rats with T1DM. At the 150 mg kg^{-1} dose, the AF reduced triglyceride levels to values lower than those observed for the control group. Furthermore, 150 mg kg^{-1} AF decreased triglyceridemia in the plasma of non-diabetic animals.

Regarding oxidative stress parameters in the plasma of T1DM animals, AF partially reversed the increase in TBA-RS levels (Figure 5a) at the dose of 100 mg kg^{-1} and completely reversed the increase in TBA-RS at the dose of 150 mg kg^{-1} ($F_{9.50}$ = 118.875; p < 0.001). There was a total reversal of the decrease in CAT (Figure 5d) activity at doses of 100 and 150 mg kg^{-1} ($F_{9.50}$ = 34.942; p < 0.001) and in SOD (Figure 5e) activity at the doses of 50, 100, and 150 mg kg^{-1} ($F_{9.48}$ = 36.263; p < 0.001). Regarding the activity of GSH-Px (Figure 5f), the AF partially reversed the reduction caused by T1DM at the dose of 100 mg kg^{-1} and completely abrogated this decrease at the dose of 150 mg kg^{-1} ($F_{9.47}$ = 22.427; p < 0.001). The AF did not change the total content of sulfhydryls (Figure 5b) (F5.30 = 0.935; p > 0.05) and of carbonyl proteins (Figure 5c) (F5.30 = 1.248; p > 0.05).

In kidneys, AF (Figure 6) reversed the increase in TBA-RS levels (150 mg kg^{-1}) ($F_{9.48}$ = 40.749; p<0.001) and in carbonyl protein content (100 and 150 mg kg^{-1}) ($F_{9.49}$ = 50.254; p<0.001) caused by T1DM. The AF (150 mg kg^{-1}),

by itself, decreased TBA-RS levels. Increase in the activity of the antioxidant enzymes SOD (E) ($F_{9.50}$ = 36.856; p<0.001) and of GSH-Px (F) ($F_{9.50}$ = 20.162; p<0.001), caused by Alloxan, was reversed, respectively, by the doses of 150 mg kg^{-1} and 100 and 150 mg kg^{-1} of *Myrcia pubipetala* AF. The total sulfhydryl content (B) ($F_{5.29}$ = 0.386; p>0.05) and catalase activity (CAT) (D) ($F_{5.30}$ = 0.222; p>0.05) were not altered by AF.

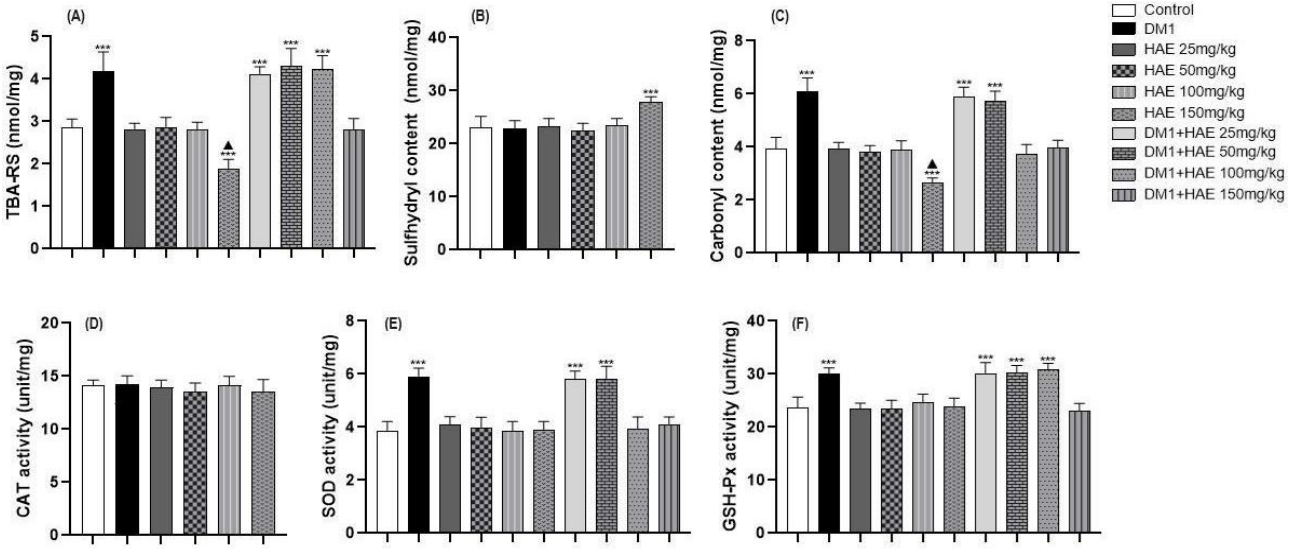

Figure 3. Effects of the chronic administration of the hydroalcoholic crude extract (HAE) of *Myrcia pubipetala* (Ep), in the presence or absence of alloxan-induced diabetes, on thiobarbituric acid reactive substances (TBA-RS) (A), total sulfhydryl content (B), total carbonyl protein content (C), catalase activity (CAT) (D), superoxide dismutase (SOD) (E) and glutathione peroxidase (GSH-Px) (F) in rat kidneys. TBA-RS is expressed as nmol of malondialdehyde per mg of protein. The total sulfhydryl content is expressed as nmol of TNB per mg of protein. The total carbonyl protein content is expressed in nmol of carbonyl per mg of protein. Enzyme activity is expressed in units per mg of protein. Data correspond to mean ± SD for 6-7 independent experiments (animals) performed in duplicate. Unlike the control, ***p < 0.001; different from the diabetic group, ▲ p < 0.001 (Multiple Duncan Test). HAE: hydroalcoholic crude extract; T1DM: Type 1 Diabetes Mellitus.

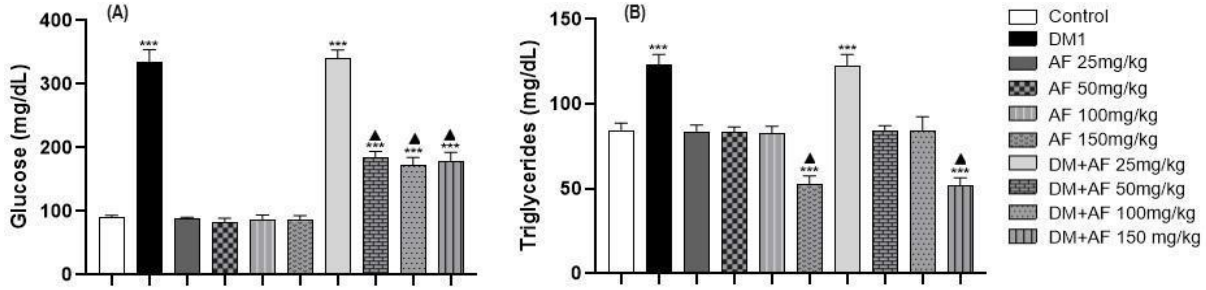

Figure 4. Effects of the chronic administration of the aqueous fraction (AF) of *Myrcia pubipetala* (Ep), in the presence or absence of alloxan-induced diabetes, on plasma levels of glucose (A) and triglycerides (B). Blood glucose and triglycerides are expressed in mg dL^{-1}. The data correspond to the mean ± SD for 6 independent experiments (animals), carried out in duplicate. Unlike the control, ***p < 0.001; different from the diabetic group, ▲ p < 0.001 (Multiple Duncan Test). AF: Aqueous fraction; T1DM: Type 1 Diabetes Mellitus.

Results demonstrate that alloxan-induced T1DM led to lipid peroxidation, protein damage, and changes in the activities of antioxidant enzymes in the blood and kidneys of rats. In contrast, HAE showed a protective effect on lipids and proteins, as evidenced by its ability to reduce TBA-RS levels, increase sulfhydryl content, and decrease the content of carbonylated proteins. Moreover, the chronic administration of HAE was able to reverse the oxidative stress parameters altered by T1DM, indicating its potential as an antioxidant (Aksenov & Markesbery, 2001).

Even though numerous studies report that the family Myrtaceae sp. have anti-inflammatory and antioxidant activities, the genus *Myrcia* DC, including the *Myrcia pubipetala* plant, has been explored in few studies employing models, such as that of the present study to date (Harbone & William, 2000; Salvador, Lourenco, Andreazzam, Pascoal, & Stefanello, 2011; Albuquerque, Heleno, Oliveira, Barros, & Ferreira, 2021). According to Salvador et al. (2011), species of the Myrtaceae family contain various types of phenolic substances that are considered antioxidants due to the donation of a hydrogen atom and/or an electron by the molecules to free radicals, causing the breakdown of the oxidation chain reaction. Anti-inflammatory,

antimicrobial, and antiproliferative activities have also been associated with these compounds (Albuquerque et al., 2021). The phenolic class is divided into flavonoids, which have high antioxidant activity, as observed in the present study (Harbone & William, 2000).

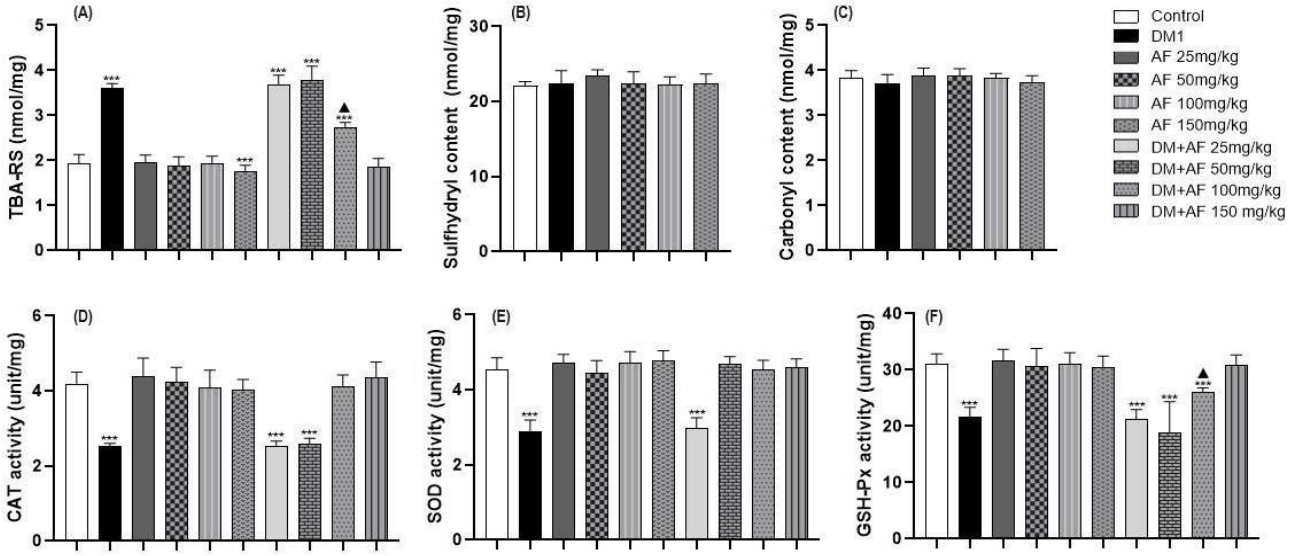

Figure 5. Effects of the chronic administration´s Effects of the aqueous fraction (AF) of *Myrcia pubipetala* (Ep), in the presence or absence of alloxan-induced diabetes, on thiobarbituric acid reactive substances (TBA-RS) (A), total sulfhydryl content (B), total carbonyl protein content (C), catalase (CAT) activity (D), superoxide dismutase (SOD) (E) and glutathione peroxidase (GSH-Px) (F) in rat blood. TBA-RS is expressed as nmol of malondialdehyde per mg of protein. The total sulfhydryl content is expressed as nmol of TNB per mg of protein. The total carbonyl protein content is expressed in nmol of carbonyl per mg of protein. Enzyme activity is expressed in units per mg of protein. Data correspond to the mean ± SD for 6-7 independent experiments (animals), performed in duplicate. Unlike the control, ***p < 0.001; different from the diabetic group, ▲ p < 0.001 (Multiple Duncan Test). AF: aqueous fraction; T1DM: Type 1 Diabetes Mellitus.

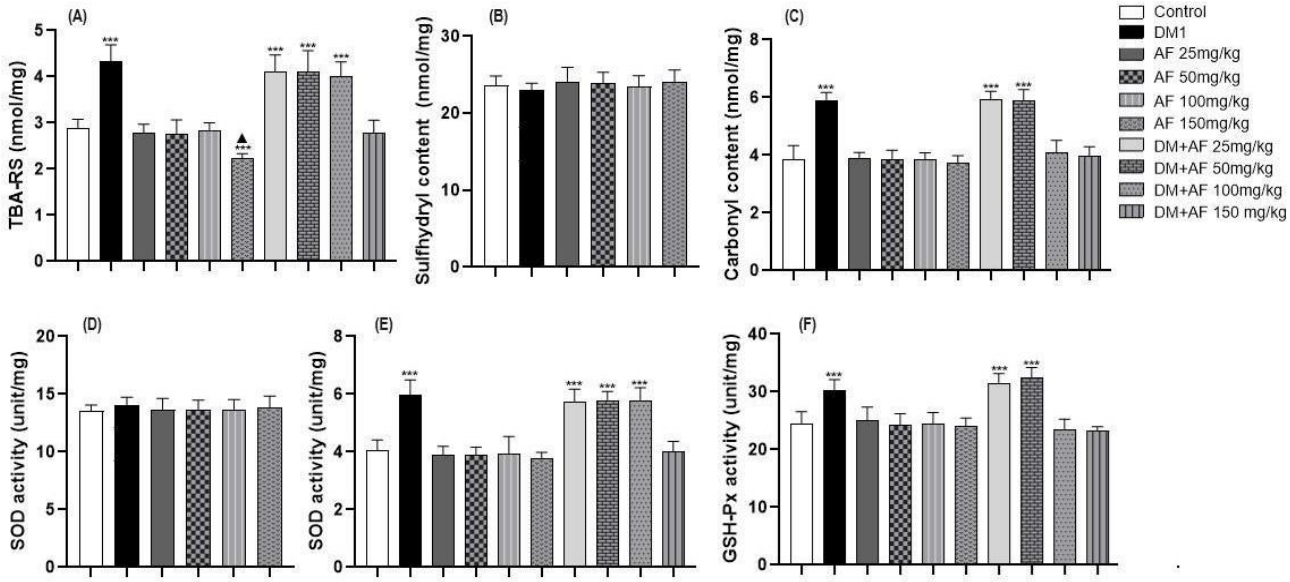

Figure 6. Effects of the chronic administration of the aqueous fraction (AF) of *Myrcia pubipetala* (Ep), in the presence or absence of alloxan-induced diabetes, on thiobarbituric acid reactive substances (TBA-RS) (A), total sulfhydryl content (B), total carbonyl protein content (C), catalase (CAT) activity (D), superoxide dismutase (SOD) (E) and glutathione peroxidase (GSH-Px) (F) in rat kidneys. TBA-RS is expressed as nmol of malondialdehyde per mg of protein. The total sulfhydryl content is expressed as nmol of TNB per mg of protein. The total carbonyl protein content is expressed in nmol of carbonyl per mg of protein. Enzyme activity is expressed in units per mg of protein. Data correspond to the mean ± SD for 6-7 independent experiments (animals), performed in duplicate. Unlike the control, ***p < 0.001; different from the diabetic group, ▲ p < 0.001 (Multiple Duncan Test). AF: aqueous fraction; T1DM: Type 1 Diabetes Mellitus.

The hypoglycemic and lipid-lowering action of HAE and AF from *Myrcia pubipetala* leaves on DM was consistent with the results of several other studies that have analyzed the abilities of isolated phenolic compounds to reduce glycemia and triglyceridemia in diabetic rats (Ong & Khoo, 2000; Vessal, Hemmati, & Vasei, 2003; Liu, Liou, Lan, Hsu, & Cheng, 2005). In accordance with our results Ferreira et al. (2011) observed

decreased triglyceride levels in rats subjected to a high-fat diet and treated with extracts from *Myrcia multiflora*. Medeiros et al. (2021) reported hypoglycemic and lipid-lowering effects of dichloromethane extracts from leaves of *Myrcia splendens*, in an animal model of T2DM. The authors also reported decreased plasma levels of triglycerides and cholesterol, both in control animals and in diabetic animals treated with *M. bella* extract, which agrees with our study.

AF extracts from *Myrcia pubipetala* at the two highest concentrations tested also significantly reversed the increase in oxidative lesions and the decrease in the antioxidant defenses in both blood and kidney of diabetic rats, evidencing its antioxidant effects possibly by modulating endogenous antioxidant defenses in animals and/or by direct antioxidant effects of components of the extract. As demonstrated by Moresco et al. (2014) and Magina et al. (2010), extracts from the species *Myrcia splendens*, *Myrcia palustris*, *Eugenia catharinae,* and *Psidium cattleyanum,* and AF from other plants of the Myrtaceae family possess many kinds of phenolic substances and flavonoids, and the amount of these compounds is directly related to their antioxidant capacity. Figueirôa et al. (2013) also demonstrated high levels of phenolic compounds and high radical scavenging power in AF from *Eugenia uniflora* and *Eugenia malaccensis*, belonging to the Myrtaceae family. Echterhoff (2018) identified different phenolic compounds in *Myrcia pubipetala*, such as gallic acid, isoquercitrin, aromadendrin, p-coumaric acid, vanillin, protocatechuic acid, chlorogenic acid, and salicylic acid, which possess essential antioxidant action. Therefore, the antioxidant effects in diabetic rats seen in our study can be related to such compounds in HAE and AF extracts.

According to Degáspari and Waszczynskyj (2004), the presence of an expressive number of phenolic compounds with antioxidant properties contributes to inhibiting lipid oxidation and other biomolecules. Phenolic compounds can scavenge free radicals, inhibit the production of inflammatory mediators, and inhibit the growth of microorganisms (Gutiérrez-Grijalva et al., 2018; Song et al., 2020; Jiang et al., 2022). These compounds can also have other therapeutic properties achieving pain relief and reducing fever, and for this reason, they have been widely studied for their potential health benefits, including antioxidant, anti-inflammatory, and anti-cancer properties, and potential effects on glucose metabolism and insulin sensitivity (Randjelović et al., 2015; Orfali et al., 2016; Pei, Ou, Huang, & Ou, 2016; Tajik, Tajik, Mack, & Enck, 2017). This antioxidant action probably favors the hypoglycemic and lipid-lowering actions of the plant *Myrcia pubipetala*.

Conclusion

The results from this study showed that the HAE and AF from *Myrcia pubipetala* caused hypoglycemic and lipid-lowering effects and antioxidant actions that are probably mediated by phenolic compounds. These actions protect the organism against oxidative stress-mediated damage from persistent hyperglycemia. These results are promising with respect to the use of *Myrcia pubipetala* as an adjuvant treatment in the control of T1DM.

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