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RESEARCH PAPERS

Hypoglycemic and antioxidant potential of *Myrcia splendens* extract in diabetic rat models

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Highlights

- EAE from Myrcia splendens reduces hyperglycemia and hypertriglyceridemia in diabetic rats
- EAE reverses oxidative stress by modulating antioxidant enzymes in blood and kidneys
- Phenolic compounds like gallic and ellagic acids contribute to EAE's hypoglycemic effects
- EAE shows potential as an adjunct treatment for diabetes-related oxidative damage

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KEYWORDS
Diabetes *Mellitus;*
Oxidative stress;
Plant extract;
Hyperglycemia;
Hypertriglyceridemia.

Abstract: We verified the effects of the subchronic administration of the ethyl acetate extract (EAE), acquired through the leaves of *Myrcia splendens*, on the changes caused by DMI and DMII induced by the administration of alloxan and streptozotocin-nicotinamide, respectively, on hyperglycemia, hypertriglyceridemia (HTG) and oxidative stress in the blood and kidneys of rats. The results showed increased levels of glucose and triglycerides in both models. In the DMI and DMII models, the EAE partially reversed the hyperglycemia and completely reversed the HTG. Alloxan-induced DMI increased TBA-RS and decreased CAT, SOD, and GSH-Px activities. Streptozotocin-nicotinamide-induced DMII increased TBA-RS, reduced total sulfhydryl content, increased CAT, and decreased GSH-Px activities in the blood of rats. Subchronic administration

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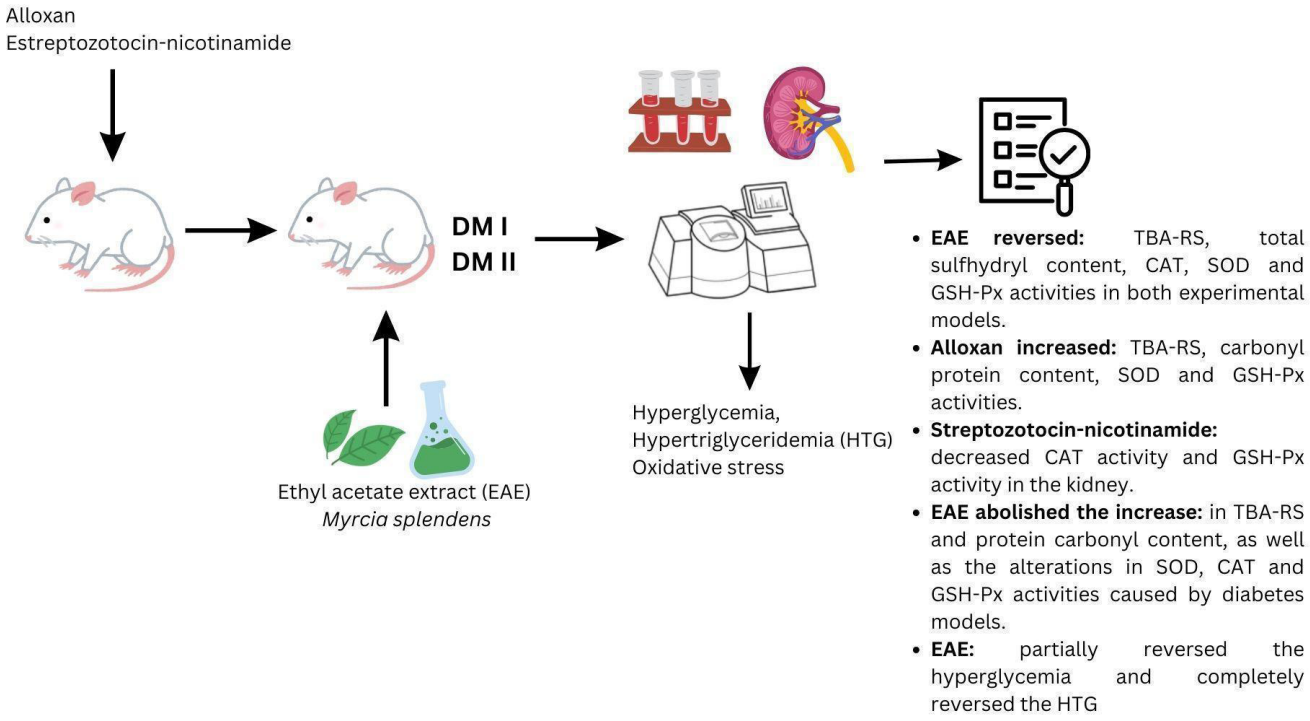
of EAE reversed the increase in TBA-RS levels, the reduction of total sulfhydryl content, and the alterations in CAT, SOD, and GSH-Px activities in both experimental models. Otherwise, alloxan increased TBA-RS levels, carbonyl protein content, SOD and GSH-Px activities, and streptozotocin-nicotinamide decreased CAT activity, as well as GSH-Px activity in the kidney. Additionally, treatment with EAE abolished the increase in TBA-RS and protein carbonyl content, as well as the alterations in SOD, CAT, and GSH-Px activities caused by diabetes models. Finally, the effects of the subchronic administration of EAE are inherent to the presence of phenolic compounds, such as gallic and ellagic acids, in the extract. Further studies are needed to identify whether EAE obtained from *Myrcia splendens* should be used as an adjuvant treatment in individuals with DM in order to minimize adverse effects caused by currently used medications.

Graphical Abstract



Introduction

Diabetes is a chronic disease characterized by high morbidity and mortality due to complications from microvascular and macrovascular disorders, such as diabetic retinopathy, chronic renal failure, and myocardial infarction, which adversely affect life quality and expectancy (Kanter & Bornfeldt, 2016). Hyperglycemia, the primary feature of diabetes, occurs from impaired insulin secretion or action, compromising the endothelium's protective functions against oxidative stress and inflammation (Lorenzi & Cagliero, 1991; Domingueti et al., 2016). Diabetes is classified into two main types: type 1 diabetes mellitus (DMI), which involves autoimmune destruction of insulin-producing cells (American Diabetes Association, 2013), and type 2 diabetes mellitus (DMII), characterized by insulin resistance and insufficient hormone release, often remaining asymptomatic until diagnosis (American Diabetes Association, 2013).

Oxidative stress occurs because of the disharmony between the formation of free radicals and the antioxidant system, increasing peripheral insulin sensitivity and boosting the functioning of DMII through molecular mechanisms (Yaribeygi et al., 2020). Free radicals are essential physiological constituents in biological homeostasis; however, oxidative stress occurs when their formation rises above the antioxidant system (Yaribeygi et al., 2018a); this is a crucial mechanism for diabetes-related disorders and insulin resistance (Yaribeygi et al., 2019, 2018c), generating pathophysiological, as well as molecular events and activating a series of deleterious pathways that drive towards insulin resistance and DM (Yaribeygi et al., 2018b; Hurrle & Hsu, 2017).

Recent intervention regulations propose using metformin as the first-choice treatment for DMII patients, in line with changes in lifestyle habits (Inzucchi & Matthews, 2015). However, if glycemic management persists, metformin is indicated in combination with another hypoglycemic agent, such as sulfonylureas, thiazolidinediones, sodium/glucose-2 cotransport enzyme inhibitors, dipeptidyl peptidase 4 inhibitors, GLP-1 receptor agonists and others (Inzucchi & Matthews, 2015; American Diabetes Association, 2017). In DMI patients, insulin treatment aims to provide insulin that mimics physiological insulin secretion. Insulin administration is usually used by subcutaneous injection and is given through syringes, pens, or insulin pumps (Johnson, 2019; Hemmingsen et al., 2021). Patients with DMI aim to reach glycemic levels close to physiological and admit flexibility regarding the time, type, and amount of food intake that can be controlled by structured education projects for DMI patients (Johnson, 2019; Pillay et al., 2015).

Therefore, the predominant therapy incorporates a series of elements, such as food, medication combination and administration, and physical exercise. There is a remarkable variety of medications available. However, the restrictions shown by the current medications, the adverse effects and the problematic adherence of the patient to the treatment denote the importance of new studies regarding the possibility of new medications with similar objectives but with fewer adverse effects and more excellent coverage (Arumugam et al., 2013).

Myrcia splendens Arumugam (M. splendens) is a small to medium-sized tree species native to neotropical regions, mainly in Brazil's southeast. This species belongs to the Myrtaceae family, which includes several plants known for their medicinal properties. *M. splendens* thrives in diverse habitats, from lowland rainforests to savannas, and is recognized for its dense foliage and small, fragrant flowers that attract various pollinators. The leaves of *M. splendens* are particularly noted for their glossy appearance and are a rich source of bioactive compounds, including polyphenols, flavonoids, and other antioxidants (Murray-Smith et al., 2009; Cascaes et al., 2015; Sobral, 2015).

In this context, the corresponding methanolic extract of *Myrcia splendens* has a high antioxidant potential, explained by the polyphenols present in its composition (Scio et al., 2012). Furthermore, butanolic components and ethyl acetate extracts from *M. splendens* have a significant proportion of phenolic substances and flavonoids, which also favors their redox character (Moresco et al., 2014; Gülçin et al., 2010). Similar to *Myrcia pubipetala*, different botanical species, such as *Myrcia splendens*, have proven to present significant results in the adjuvant treatment of diabetes by demonstrating pharmacological effects by acting on gene expression associated with glucose metabolism, mitigating oxidative stress and inflammation, and improving endothelial function (Orgah et al., 2020) and some species of the *Myrcia* genus are commonly referred to as 'vegetable insulin' (Basting et al., 2014). In this aspect, Myrtaceae species are frequently used in traditional medicine. However, more studies are needed to understand their chemical characteristics and functions as the origin of bioactive substances (Medeiros et al., 2021).

Therefore, the objective of this work was to verify the effects of the subchronic administration of the ethyl acetate extract (EAE), acquired through the leaves of *M. splendens*, on the changes caused by DMI and DMII induced by the administration of alloxan and streptozotocin-nicotinamide, respectively, on hyperglycemia, hypertriglyceridemia and oxidative stress in the blood and kidneys of rats.

Material and methods

Obtaining plant material

Leaves of *M. splendens* (Sw.) DC, Myrtaceae were collected in Blumenau, state of Santa Catarina, Brazil (26°53'55.2 "S 49°04'41.3 "W) in October 2017. The botanist Dr. André Luiz de Gasper from the Department of Natural Sciences at the Universidade Regional de Blumenau (FURB) and Dr. Marcos Sobral from the Department of Botany at the Federal University of Minas Gerais (UFMG) verified the plant material and an exemplary voucher was left at the Herbarium Dr. Roberto Miguel Klein from the same institute under number FURB 00607.

Obtaining crude extract

The collected leaves were dried at room temperature and crushed in a knife mill. The pulverized sample was ground and macerated in ethyl acetate to obtain crude extract (EAE). The maceration technique was performed for three days, followed by filtration and repeated procedure. The extract from the two processes was mixed and concentrated on a rotary evaporator under reduced pressure until complete drying.

Animals

Male Wistar rats (230-280 g) were used for the studies, acquired from the Central Animal House of the Regional University of Blumenau, Blumenau, Brazil. The animals participating in the trial were weaned at 21 days of age. In the period before the experiment, the rats were acclimated and placed in the new environment for seven days, being exposed to the 12 h light / 12 h dark cycle, with a constant temperature of (22 ± 1 °C), with full access to commercial chow and drinking water. The rats were housed in 4 four cages, and the experiments were performed according to law nº 11794 of October 8, 2008, as well as the other teaching and research guidelines, fundamentally the norms of the National Council for Control of Animal Experimentation - CONCEA. The environmental conditions, lighting, accommodation, and nutrition recommendations followed the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1996). The Ethics Committee for Animal Research of the University of Joinville Region, Joinville, Brazil, approved the experimental protocol under CEUA protocol number 011/2017.

Experimental protocols

Analysis of phenolic compounds in the EAE by HPLC-ESI-MS/MS

Our research group already analyzed phenolic compounds in this EAE of *M. splendens*, as described by Paganelli et al.,

2020, in a Agilent® 1200 chromatograph coupled to a triple quadrupole/linear ion trap mass spectrometer Qtrap® 3200 (Applied Biosystems/MDS SCIEX, USA). Pairs of ions were monitored in multiple reaction monitoring (MRM) mode.

For recognition and quantification, 47 standard phenolic compounds were evaluated under the same circumstances specified above, including 4-aminobenzoic acid, 4-hydroxymethylbenzoic acid, apigenin, aromadendrin, caffeic acid, carnosol, catechin, chlorogenic acid, chrysin, cinnamic acid, coniferaldehyde, ellagic acid, epicatechin, epigallocatechin, epigallocatechin gallate, eriodictyol, ferulic acid, fustin, galangin, gallic acid, hispudulin, isoquercetin, kaempferol, mandelic acid, methoxyphenylacetic acid, myricetrin, naringerin, naringin, p-anisic acid, p-coumaric acid, pinocembrin, protocatechuic acid, quercetin, resveratrol, rosmarinic acid, rutin, salicylic acid, scopoletin, sinapaldehyde, sinapic acid, syringaldehyde, syringic acid, taxifolin, umbelliferone, vanillic acid and vanillin (0.02 to 6 mg L^{-1}).

Induction of DMI and treatment

We were given an alloxan injection (150 mg/kg; intraperitoneal [i.p.]) solved in 0.1 M citrate buffer (pH 4.5) to induce DMI in rats during a 12-hour fast. Citrate buffer was injected alone in control rats. After 24 hours of injection administration, the glycemic levels were analyzed employing a strip-operated blood glucose sensor G-TECH free light. The treatment began after the diagnosis of diabetes when fasting blood glucose was above 200 mg/dL (Sheela et al., 2013).

The rats were separated into the following groups (n = 7): Control group: received an i.p. injection of citrate buffer (pH 4.5) (once) and water by gavage, once daily for 15 days. DMI Group: received an i.p. injection of alloxan (diluted to 2% in 0.05 M sodium citrate solution, pH 4.5) at a dose of 150 mg/kg (once) and water via gavage, once daily for 15 days; Control group extractions: received an i.p. injection of citrate buffer (pH 4.5) (once) and EAE (25, 50, 100 or 150 mg/kg) from leaves of the species of *M. splendens* by gavage, once a day for 15 days. DMI + extract group: received an i.p. injection of alloxan (diluted to 2% in 0.05 M sodium citrate solution, pH 4.5) at a dose of 150 mg/kg (once) and EAE (25, 50, 100 or 150 mg/kg) through gavage, once a day for 15 days. The subchronic treatment was administered orally to the animals for 15 consecutive days (gavage), obeying 24 hours between one treatment and another.

Induction of DMII and treatment

To induce DMII, rats in a 12 h fast received a streptozotocin injection (60 mg/kg; i.p.) solved in 0.1 M citrate buffer (pH 4.5) 15 min after the nicotinamide prescription (120 mg/kg; i.p.). Citrate buffer was inoculated alone in control rats. The nicotinamide prevents the β pancreatic cells (by 40%) from the cytotoxicity of streptozotocin and triggers DMII (Sheela et al., 2013). After 72 h, the glycemic levels were dosed using a strip-operated blood glucose sensor G-TECH free light. The treatment began after confirmation of diabetes when fasting blood glucose was more significant than 200 mg/dL (Sheela et al., 2013).

The rats were divided into groups (n = 7) in the following way: Control group: received water via i.p. injection (once) and water orally (by gavage) once a day for 15 days; DMII group: received an i.p. injection of streptozotocin (60 mg/kg) solved in 0.1 M citrate buffer (pH 4.5), 15 min after the nicotinamide prescription (120 mg/kg; i.p.) (once) and water by gavage, once a day during 15 days; Control groups extract: received an i.p. injection of water (once) and EAE (25, 50, 100 or 150 mg/kg) from *M. splendens* species by gavage, once a day during 15 days; DMII + extract groups: received an i.p. injection of streptozotocin (60 mg/kg) solved in 0.1 M citrate buffer (pH 4.5), 15 min after the nicotinamide prescription (120 mg/kg; i.p.) (once) and EAE (25, 50, 100 or 150 mg/kg) by gavage, once a day during 15 days subsequent for subchronic treatment, respecting a space of 24 h interval between administrations.

After this phase, for both treatments, the animals were sacrificed by decapitation without anesthesia, and blood and kidneys were collected, as well as taken for oxidative stress, triglyceride, and glucose analysis. The EAE (25, 50, 100, and 150 mg/kg) doses were selected according to studies by Kar et al. (2003) and Ravi et al. (2004, 2005).

Preparation of tissue

The animals were euthanized, and the kidneys were removed, dissected and homogenized. The homogenates were prepared using a Potter-Elvehejem homogenizer (Remi motors, Mumbai, India) by passing five pulses. They were centrifuged at 800 × g for 10 min at 4 °C to discard nuclei and cell debris. The pellet was discarded, and the supernatant was saved in aliquots and kept at -80 °C. The maximum time between the homogenate preparation and analysis was always less than three days. All procedures were performed at 0-4 °C (Lima et al., 2017).

Serum preparation

Serum was produced from total blood samples obtained from rats. The peripheral blood was collected and transferred to tubes without anticoagulant. After separation, it was centrifuged at 1,000 x g for 10 min and used for studies of biochemical parameters (Lima et al., 2017).

Preparation of erythrocytes and plasma

Whole blood was collected and stored in heparinized tubes to separate erythrocytes. Blood samples were centrifuged at 1,000 × g; the plasma was removed by aspiration and frozen at -80 °C until analysis of oxidative stress. The erythrocytes were washed three times with a cold saline solution (0.153 mol/L sodium chloride). Lysates were prepared by adding 1 mL of distilled water to 100 µL of washed and frozen red blood cells at -80 °C until the antioxidant enzyme activities were performed. To define the activity of the antioxidant enzymes, the erythrocytes were frozen and thawed three times and centrifuged at 13,500 × g for 10 min. The supernatant was diluted to around 0.5 mg/mL of protein.

Biochemical studies

Dosage of glucose and triglycerides

Glucose and triglycerides were measured using the Glucose and Triglycerides Liquiform kits (Labtest). According to the manufacturer's package insert, absorbance was verified using a UV-vis Shimadzu spectrophotometer.

Catalase assay (CAT)

CAT activity was established using Aebi's (1984) method. This method is based on the disappearance of hydrogen peroxide (H_2O_2) in a reaction medium of 25 µL of sample and 600 µL of 10 mM potassium phosphate buffer, pH 7.0, 20 mM H_2O_2. The absorbance was counted every 10 sec for 1 min and 40 sec at 240 nm using a UV-vis Shimadzu spectrophotometer. One CAT corresponds to 1 µmol of H_2O_2 consumed per minute, and the specific activity is calculated as CAT units/mg protein.

Superoxide dismutase assay (SOD)

SOD activity was analyzed by the methodology of Marklund (1985), a procedure dependent on superoxide ($O2^{\bullet-}$), 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. Then, 20 µL of pyrogallol was added, and the absorbance was counted every 30 sec for 3 min at 420 nm. Inhibition of auto-oxidation of pyrogallol occurs in the presence of SOD, the activity of which can be indirectly tested spectrophotometrically. One unit of SOD is determined 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 protein.

Glutathione peroxidase assay (GSH-Px)

GSH-Px activity was measured using Wendel's (1981) method, which used *tert*-butylhydroperoxide as a substrate. The decomposition of NADPH was controlled at 340 nm for 3 min and 30 sec. 90 µL of each sample was added to the medium containing 800 µL of buffer, 20 µL of 2.0 mM GSH, 30 µL of 0.15 U/mL GSH reductase, 10 µL of 0.4 mM azide, and 10 µL of 0.1 mM NADPH. The absorbance was counted every 10 sec for one min and thirty sec. Then, 50 µL of 0.5 mM *tert*-butylhydroperoxide was added, and the absorbance was read for two more min. One GSH-Px unit is characterized as 1 µmol of NADPH consumed per minute, and the specific activity is defined as GSH-Px units/mg of protein.

Total sulfhydryl content

The total sulfhydryl content was performed following the method of Aksenov & Markesbery (2001), based on the reduction of dithionitrobenzoic acid (DTNB) by thiols, yielding a yellow derivative (TNB), which is evaluated spectrophotometrically at 412 nm. 50 µL of homogenate was added to 1 mL of phosphate-buffered saline (PBS), pH 7.4, composed of 1 mM ethylenediaminetetraacetic acid (EDTA). The reaction started with 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 presented as nmol TNB/mg protein.

Thiobarbituric acid reactive substances (TBA-RS)

TBA-RS was defined according to the method of Ohkawa et al. (1979). The methodology measures malondialdehyde (MDA), resulting from lipoperoxidation, provided predominantly by hydroxyl free radicals. At first, plasma and kidney in 1.15% KCl were mixed with 20% trichloroacetic acid and 0.8% thiobarbituric acid and heated in a boiling water bath for 1 h. The absorbance determined TBA-RS at 535 nm. A calibration curve was acquired using 1,1,3,3-tetramethoxypropane as the MDA precursor, and each curve point was exposed to the same treatment as the supernatants. TBA-RS content was presented in nmol of MDA formed per mg of protein.

Protein carbonyl content

Protein Carbonyl content was tested using the methodology detailed by Reznick & Packer (1994), based on the reaction of protein carbonyls with dinitrophenylhydrazine to form dinitrophenylhydrazone. This yellow compound is measured spectrophotometrically at 370 nm. 200 µL of plasma or homogenate were added to plastic tubes containing 400 µL of 10 mM dinitrophenylhydrazine (prepared in 2 M HCl). Samples were kept in the dark for 1 h and vortexed every 15 min. 500 µL of 20% trichloroacetic acid was added to each tube. The mixture was vortexed and centrifuged at 14,000 x g for 3 min, and the resulting supernatant was excluded. The pellet was washed with 1 mL ethanol/ethyl acetate (1:1 v/v), vortexed, and centrifuged at 14,000 x g for 3 min. The supernatant was discarded, and the pellet was resuspended in 600 µL of 6 M guanidine (prepared in a 20 mM potassium phosphate solution, pH 2.3), pre-vortexed, and incubated at 60 °C for 15 min. Thus, samples were centrifuged at 14,000 x g for 3 min, and the supernatant was used to measure absorbance at 370 nm. Results were described as carbonyl content (nmol/mg protein).

Protein determination

Protein was established by the Lowry et al. (1951) method, using serum bovine albumin as standard.

Results

Phenolic composition of ethyl acetate extract (EAE)

After complete drying, the EAE yielded 5.4% (w w^{-1}). Ten phenolic compounds were identified in the extract from forty-seven investigated standards. The compound with the highest amount found was ellagic acid (10.68 ± 0.10 µg g^{-1}), followed by gallic acid (6.29 ±0.17 µg g^{-1}) (Table 1).

Table 1. Identified phenolic (μg g^{-1}) in *Myrcia splendens* EAE leaves.

Phenolic compound	Rt (min)	MF	TM	EM	MS/MS	EBAE
			(Da)	(M-H, *m*/*z*)	(*m*/*z*)	
Gallic acid	3.71	$C_7H_6O_5$	170.12	168.90	125.00	6.29 ± 0.17
Protocatecuic acid	6.27	$C_7H_6O_4$	154.12	152.92	109.00	0.57 ± 0.005
Syringic acid	9.91	$C_9H_{10}O_5$	198.17	196.93	121.10	0.41 ± 0.006
p-Coumaric acid	10.32	$C_9H_8O_3$	164.05	162.92	119.10	2.15 ± 0.16
Syringaldehyde	10.67	$C_9H_{10}O_4$	182.17	180.94	151.00	0.19 ± 0.08
Salicylic acid	10.75	$C_7H_6O_3$	138.12	136.94	93.00	1.66 ± 0.02
Umbelliferone	10.78	$C_9H_6O_3$	162.14	160.94	133.10	0.05 ± 0.001
Isoquercetin	10.79	$C_{21}H_{20}O_{12}$	464.38	463.15	300.00	n.q.
Coniferaldehyde	11.24	$C_{10}H_{10}O_3$	178.18	177.01	162.00	0.08 ± 0.002
Ellagic acid	11.78	$C_{14}H_6O_8$	302.19	300.95	145.00	10.68 ± 0.10

Rt = Retention time (min); MF = Molecular formula; TM = Theoretical mass (Da); EM = Experimental mass (*m*/*z*); MS/MS = MS/MS Fragments (*m*/*z*); <LOD = Less than the limit of detection; n.q. = non-quantified; EBDM = Dichloromethane extract; EBH = hydroalcoholic extract; EBAE = Ethyl acetate extract. Adapted from Paganelli et al. (2020).

Effects of subchronic administration of ethyl acetate extract (EAE) from *M. splendens* on glucose and triglyceride levels on alloxan-induced type-1 and streptozotocin-nicotinamide-induced type-2 diabetic in rats

We investigated the effects of subchronic administration of EAE (25, 50, 100, and 150 mg/kg) obtained from the leaves of *M. splendens* on glucose and triglyceride levels in the serum of DMI and DMII rats. Figure 1 shows that alloxan and streptozotocin-nicotinamide administration significantly enhanced glucose (A) [F(9.50)=829.171; p<0.001] and [F(9.52)=69.309; p<0.001], and triglyceride (B) levels [F(9.50)=90.963; p<0.001] and [F(9.52)=14.143; p<0.001], respectively, in the serum of rats, when compared to the control groups. The DMI model EAE (50, 100 e 150 mg/kg) (47.6%; 50.0% and 47.9%) and DMII model EAE (100 and 150 mg/kg) (28.9% and 54.7%) partially reversed the hyperglycemia. In addition, EAE at doses of 25 and 50 mg/kg (19.4%; 21.7% and 14.0%; 27.7%, respectively) partially reversed, and at doses of 100 and 150 mg/kg reversed the hypertriglyceridemia induced by alloxan and streptozotocin-nicotinamide administration.

Effects of the subchronic administration of EAE from *M. splendens* on the alterations in the blood oxidative stress parameters caused by alloxan-induced type-1 diabetes and streptozotocin-nicotinamide-induced type-2 diabetic in rats

We next investigated the effects of the subchronic administration of EAE (25, 50, 100 and 150 mg/kg) on the alterations in TBA-RS, total sulfhydryl content, protein carbonyl content and antioxidant enzyme activities caused by DMI and DMII in the blood of rats. As shown in Figure 2, alloxane-induced DMI elevated TBA-RS levels (A) [F(9.49)=72.435; p<0.001] in the plasma and reduced CAT (E) [F(9.50)=44.803;p<0.001], SOD (D) [F(9.49)=38.823;p<0.001] and GSH-Px (F) [F(9.44)=25.125; p<0.001] activities in the erythrocytes of diabetes-induced rats, when compared to control groups. Figure 2 also shows that the streptozotocin-nicotinamide DMII model increased TBA-RS (A) [F(9,52)=12.011; p<0.001], reduced total sulfhydryl content (B) [F(9.52)=23.397; p<0.001], increased CAT (E) [F(9.52)=8.252; p<0.001] and reduced GSH-Px (F) [F(9.52)=7.941; p<0.001] activities. *Post hoc* analysis showed that EAE, *per se*, in both models, at doses of 100 and 150 mg/kg increased the total sulfhydryl content (B) and SOD activity (E). In the DMI, at doses of 50, 100 and 150 mg/kg, reduced protein carbonyl content (C) and in the DMII, at doses of 100 and 150 mg/kg, "*per se*" reduced protein carbonyl content (C), when compared to the control groups in the blood of rats. The subchronic administration of EAE (100 and 150 mg/kg) reversed the increase in TBA-RS levels (A) and the reduction in CAT (50, 100 and 150 mg/kg) (E), SOD (D) and GSH-Px activities (100 and 150 mg/kg) (F) caused by DMI, in the blood of rats. Moreover, subchronic administration of EAE at doses of 100 and 150 mg/kg partially reversed the increases in TBA-RS levels (19.2% and 19.2%) (A), at a dose of 50 mg/kg partially reversed (23.8%), at 100 mg/kg reserved the reduction and at 150 mg/kg reserved and increased sulfhydryl content in the blood of rats. Regarding the antioxidant enzyme CAT (E), subchronic administration of EAE at doses of 50, 100 and 150 mg/kg reversed the increase in the CAT activity. EAE treatment at doses of 25 and 50 mg/kg partially reversed (24.0% and 26.9%) and at doses of 100 and 150 mg/kg reversed GSH-Px reduction (F) when administered in animals that received streptozotocin-nicotinamide.

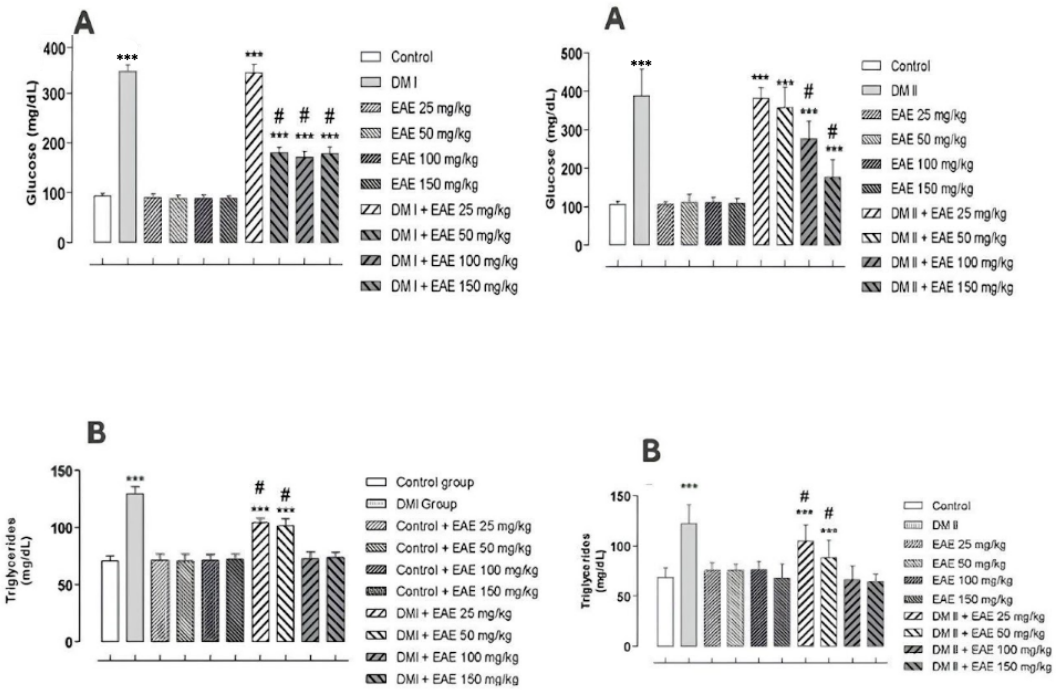


Figure 1. Effect of different EAE doses (25, 50, 100, or 150 mg/kg) on the effects elicited by DMI and DMII on glucose levels (A) and triglycerides levels (B) in the blood of 60-day-old Wistar rats. Results are expressed as mean ± standard deviation for 7-8 independent (animal) experiments performed in duplicate. ***p <0.001, compared to the control; #Partially reversed.

The effects of the subchronic administration of EAE from *M. splendens* on the alterations in the kidney oxidative stress parameters caused by alloxan-induced type-1 diabetes and streptozotocin-nicotinamide-induced type-2 diabetes in rats

We also investigated the effects of the subchronic administration of EAE (25, 50, 100, and 150 mg/kg) on the alterations in oxidative stress parameters induced by DMI and DMII in the kidneys of rats. Figure 3 shows that alloxan increased TBA-RS levels (A) [$F(9.50)=77.549$; $p<0.001$], protein carbonyl content (C) [$F(9.45)=64.891$; $p<0.001$], SOD (D) [$F(9.49)=43.472$; $p<0.001$], and GSH-Px (F) [$F(9.49)=41.478$; $p<0.001$] activities and that streptozotocin-nicotinamide decreased CAT (E) [$F(9.52)=20.126$; $p<0.001$] and GSH-Px (F) activities [$F(9.52)=22,956$; $p<0.001$], when compared to the control groups. The subchronic administration of the EAE (100 and 150 mg/kg) abolished the increased TBA-RS levels (A) and protein carbonyl content (C), as well as the enhanced SOD (E) and GSH-Px (150 mg/kg) (F) activities in the kidneys caused by DMI. Additionally, EAE treatment at doses of 100 and 150 mg/kg reversed CAT reduction (E), and at 150 mg/kg, reversed GSH-Px reduction (F) caused by DMII.

Discussion

Research with experimental models of diabetes in rodents is significantly used to demonstrate the symptomatology of diabetes, and the compounds commonly applied are alloxan and streptozotocin (Vareda et al., 2014). As a consequence, streptozotocin can cause polyphagia, polydipsia, and polyuria, as well as weight reduction in rodents induced by diabetes, as seen in the study by Vareda et al. (2014). Alloxan, chemically called 5,5-dihydroxyl pyrimidine-2,4,6-trione, is a carcinogen and cytotoxic glucose analog. Alloxan is commonly used to analyze the antidiabetic potential of pure compounds and plant extracts in diabetes studies (Ighodaro et al., 2018). Therefore, alloxan and streptozotocin are the main diabetogenic compounds used to verify test compounds' antidiabetic or hypoglycemic capacity (Ighodaro et al., 2018).

M. is one of the notable genera of the Myrtaceae family. Certain species can be applied in folk medicine, such as the group "pedra-hume-caá" or "vegetable insulin" (insulin plant) that is used in the treatment of diabetes. Furthermore, the anti-inflammatory, antinociceptive, antioxidant, and antimicrobial action are associated with *M.* essential oils, as the hypoglycemic, anti-hemorrhagic, and antioxidant functions are related to the extracts (Cascaes et al., 2015). Triterpenes, steroids, flavonoids, flavonoid glycosides, and acetophenone products in *M.* allow different activities (Ferreira et al., 2014). In this context, flavonoid glycosides and acetophenone products indicated the inhibition of aldose reductase and α-glucosidase, which would elucidate the conventional use of *M.* species in managing diabetes (Cascaes et al., 2015). Furthermore, the antimicrobial and anti-inflammatory actions are functions verified for other compounds isolated from *M.* (Cascaes et al., 2015). In an animal model of DMII, the dichloromethane extract from *M. splendens* reduced glycemia and triglycerides. It demonstrated remarkable antioxidant action against oxidative stress markers. It improved the activity of endogenous antioxidant enzymes, such as CAT, probably due to the presence of phenolic compounds in the

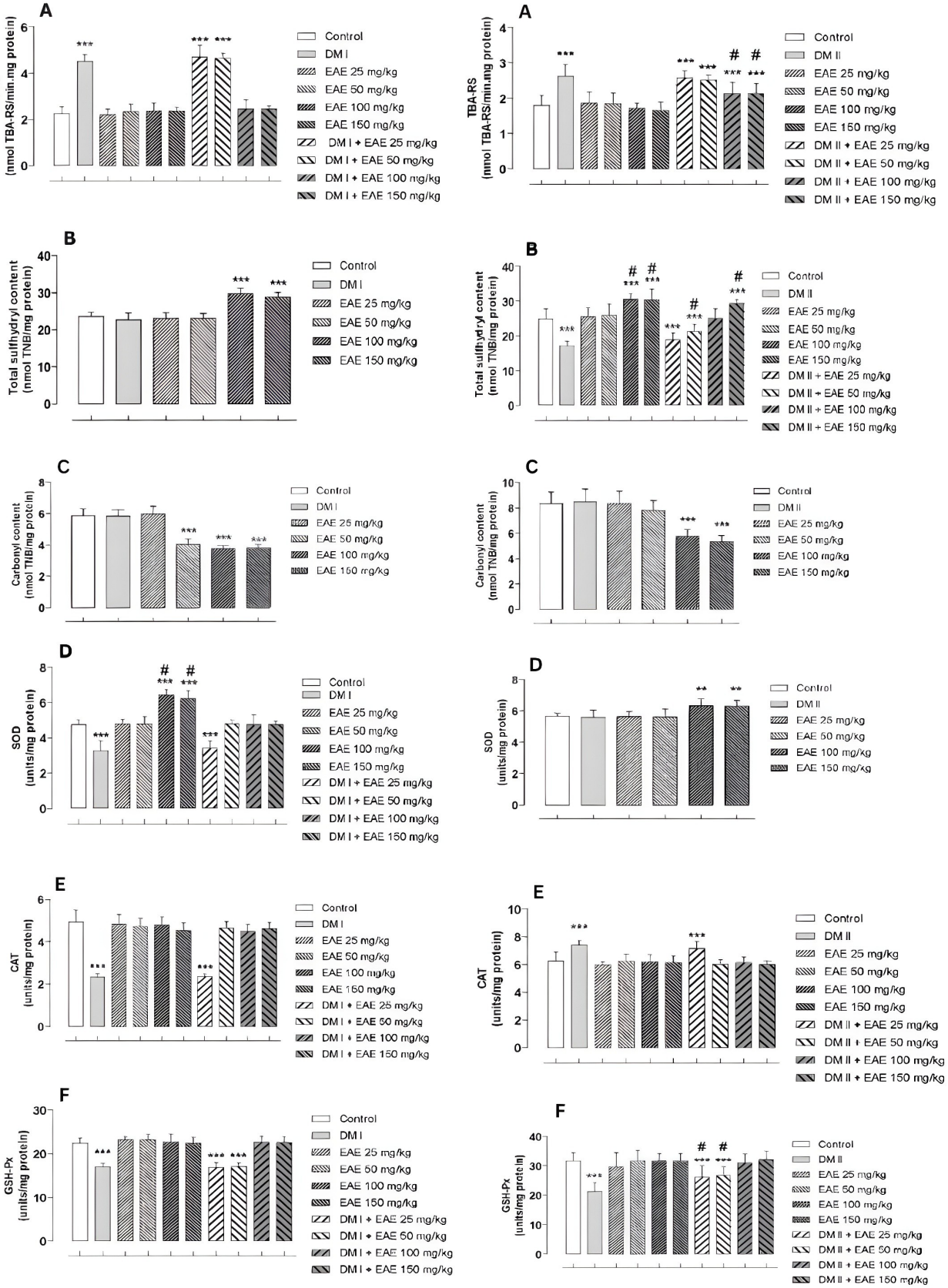


Figure 2. Effect of different EAE doses (25, 50, 100, or 150 mg/kg) on the effects elicited by DMI and DMII on TBA-RS (A) levels, total sulfhydryl content (B), protein carbonyl content (C), SOD (D), CAT (E) and GSH-Px (F) activities in the blood of 60-day-old Wistar rats. Results are expressed as mean ± standard deviation for seven independent (animal) experiments performed in duplicate. ***p<0.001 and **p<0.001, compared to controls; #Partially reverse or potentiation.

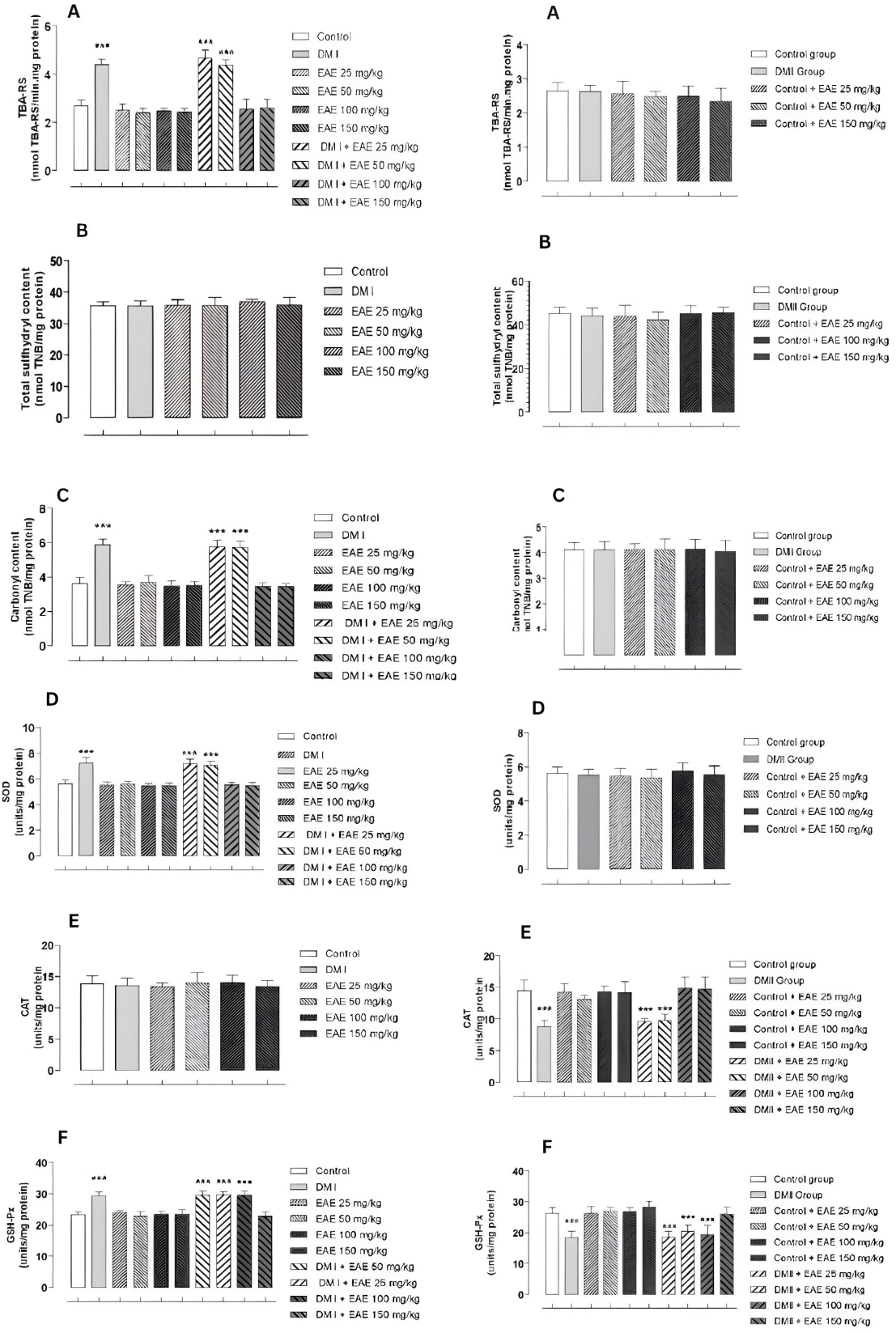


Figure 3. Effect of different EAE doses (25, 50, 100, or 150 mg/kg) on the effects elicited by DMI and DMII on TBA-RS (A) levels, total sulfhydryl content (B), protein carbonyl content (C), SOD (D), CAT (E) and GSH-Px (F) activities in the kidney of 60-day-old Wistar rats. Results are expressed as mean ± standard deviation for seven independent (animal) experiments performed in duplicate. ***p<0.001, compared to the control.

extract (Medeiros et al., 2021). Also, Moresco et al. (2014) verified, *in vitro*, that ethyl acetate and n-butanol from the hydroalcoholic extract of *M. splendens* and *M. palustris* have an antioxidant effect in DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and iron reduction test, supposedly due to the presence of myricitrin, the main compound found in the fractions of both species. According to Paganelli et al. (2020), the compounds identified in the phenolic composition of the EAE were gallic acid (GA), protocatechuic acid, syringic acid, p-coumaric acid, syringaldehyde, salicylic acid, umbelliferone, isoquercitrin, coniferaldehyde and ellagic acid (EA). The compound with the highest amount found was EA, followed by gallic acid.

The GA has a potent antioxidant and free radical scavenging action, which can modulate inflammation, apoptosis, and oxidative stress in various pathophysiological conditions. It also has hypoglycemic activity through its antioxidant and anti-inflammatory effects (D'Souza et al., 2014). According to Xu et al. (2021), GA therapy decreases serum creatinine, blood urea nitrogen, albumin levels, and TGF-ß1 expression in rat models with DMI nephropathy. According to Wong et al. (2022), treatment with GA and andrographolide improves diabetes complications through pancreatic islet regeneration and renal and hepatic defense against tissue damage. EA is characterized by being a natural compound identified in different fruits and vegetables and has antibacterial and antifungal effects, ranging from anti-inflammatory protection to antidiabetic, hepatoprotective, and cardioprotective (Aishwarya et al., 2021; Diao et al., 2022; Päivärinta et al., 2006; Zahoor et al., 2020; Evtyugin et al., 2020; Kábelová et al., 2021; Ríos et al., 2018). In this context, phenolic compounds, such as GA and EA, play a significant role in diabetes management due to their potent antioxidant and anti-inflammatory properties. These compounds help mitigate the oxidative stress associated with hyperglycemia, a critical factor in the progression of diabetes and its complications. GA, commonly found in various fruits, teas, and nuts, has improved insulin sensitivity and reduced blood glucose levels by enhancing gene expression in glucose uptake and metabolism. Additionally, GA can inhibit the formation of advanced glycation end-products (AGEs), which are harmful compounds formed when proteins or lipids become glycated as a result of exposure to sugars, thereby reducing the risk of diabetic complications such as neuropathy and retinopathy (Zhang et al., 2019; Mahmoud et al., 2019). Similarly, EA, a natural polyphenol found in berries, pomegranates, and nuts, contributes to diabetes management by modulating pathways related to glucose metabolism and inflammatory responses. EA has been shown to decrease blood glucose levels and improve pancreatic ß-cell function, which is crucial for insulin secretion. Moreover, it exhibits anti-inflammatory effects by downregulating pro-inflammatory cytokines and inhibiting pathways like NF-κB, often activated in diabetic conditions. These combined effects make EA a promising compound for reducing insulin resistance and protecting against the vascular and neurological complications of diabetes (Bhadri et al., 2020; Umesalma & Sudhandiran, 2010). According to Naraki et al. (2023), the administration of EA (10 mg/kg/day, orally) and repaglinide in DM2 rats decreased glucose, fructosamine, aldose reductase, triglycerides, total cholesterol, fatty acids, aspartate transaminase, alanine transaminase, gamma-glutamyl transferase, total bilirubin, TNF-α, IL-6, as well as increased levels of GSH, CAT, SOD and adiponectin. In addition, administration of EA (0.2% and 2% in the diet) to diabetic rats prevented glycation-mediated red blood cell-immunoglobulin G cross-linking, hemoglobin A1c, and N-carboxymethyl lysine accumulation, which are results of advanced glycation found mainly in the diabetic kidney (Naraki et al., 2002; Raghu et al., 2016).

The results presented in this study, in the DMI and DMII models, EAE (50, 100, and 150 mg/kg) and (100 and 150 mg/kg) partially reversed hyperglycemia. The hypoglycemic action reduces the formation of free radicals, preventing other dysfunctions caused by diabetes. Furthermore, EAE at doses of 25 and 50 mg/kg partially reversed, and at doses of 100 and 150 mg/kg completely reversed the HTG in both diabetes models, contributing to the reduction of cardiovascular diseases associated with DM and HTG.

Understanding triglyceride (TG) metabolism is critical to recognize when the intervention of HTG should be applied, aiming to prevent subsequent damage associated with DM (Valaiyapthi et al., 2019). Current studies addressing HTG have shown the relationship between high levels of TG and increasing morbidity and mortality in cardiovascular diseases, making TG a marker for atherosclerosis (Budoff, 2016). Furthermore, HTG and the reduction in high-density lipoprotein (HDL-C) levels correlate with metabolic pathologies and DM (Budoff, 2016; Li et al., 2017; Wiesner & Watson, 2017). The research by Vareda et al. (2014) proposes that the flavonoids present in the extract of *M. bella*, when administered to mice, alter the lipid metabolism by reducing TG and cholesterol levels, showing the lipid-lowering activity of *M.*

According to research by Custodio & Lima (2018), ethyl acetate extract from *M. splendens* contains antioxidant and hypoglycemic action in rats, preventing hyperglycemia and minimizing oxidative stress. Similarly, the study conducted by Medeiros et al. (2021) demonstrated that the administration of streptozotocin-nicotinamide in rats induces DMII, HTG and oxidative stress in the blood and kidneys. In the same study, it was demonstrated that the subchronic administration of *M. splendens* dichloromethane extract, which contains EA, protocatechuic acid, syringic acid, p-coumaric acid, salicylic acid, isoquercetin ferulic acid, umbelliferone, conifer aldehyde, cinnamaldehyde and carnosol, partially reversed hyperglycemia, reversed HTG, decreased lipid peroxidation, protein damage, as well as, reversed changes in the antioxidant activity of enzymes.

Regarding the alterations in the blood oxidative stress parameters, the subchronic administration of EAE (100 and 150 mg/kg) reversed the increase of TBA-RS levels and the decrease of CAT (50, 100, and 150 mg/kg), SOD, and GSH-Px activities (100 and 150 mg/kg) caused by DMI, in the blood of rats. In the DMII model, the subchronic administration of EAE at doses of 100 and 150 mg/kg partially reversed the increases in TBA-RS levels, at 50 mg/kg partially reversed, at 100 mg/kg reserved the reduction and at 150 mg/kg reserved and increased sulfhydryl content in the blood of rats. Moreover, the subchronic administration of EAE at doses of 50, 100 and 150 mg/kg reversed the increase in CAT activity, at doses of 25 and 50 mg/kg partially reversed, and at doses of 100 and 150 mg/kg reversed the reduction of GSH-Px activity. Due to its antioxidant activity, the extract reduced the free radicals, preventing lipid peroxidation, protein carbonylation, and the

reduction of sulfhydryl content, thus preventing blood oxidative damage and its dissipation to other parts of the body. Carbonyl groups can be formed when ROS act directly with proteins or with sugars and lipids, forming strongly reactive carbonyl compounds that begin to interact with proteins. Given this, the determination of carbonyl protein content is notably disclosed to evaluate the protein oxidative content (Berlett & Stadtman, 1997). Lipid peroxidation characterizes them by the process where free radicals and ROS remove electrons from lipids and then form reactive intermediate products that can continue a series of reactions (Su et al., 2019). Peroxidation products can be responsible for the proliferation of ROS or cause degrading reactions with essential molecules, such as proteins and DNA (Gaschler & Stockwell, 2017).

Regarding renal oxidative stress, the subchronic administration of EAE (100 and 150 mg/kg) abolished the increase in TBA-RS levels and carbonyl protein content, as well as the increase in SOD and GSH-Px activities (150 mg/kg) caused by the DMI model. Additionally, treatment with EAE at doses of 100 and 150 mg/kg reversed the reduction in CAT and at 150 mg/kg, the decrease in GSH-Px caused by the DMII model, showing that EAE also had an antioxidant effect, probably through the reduction of free radicals and ROS, minimizing the renal damage generated by the oxidative stress induced by hyperglycemia in both models of diabetes.

Therefore, this research showed that the subchronic administration of EAE has hypoglycemic and hypolipidemic effects and marked antioxidant capacity in DMI and DMII models in rodents. The EAE minimized lipid peroxidation and protein damage and prevented changes in antioxidant enzyme activities in the blood and kidneys of rats, demonstrating antioxidant capacity. The data showed that the action of GA and EA, the main phenolic compounds present in the extract, probably mediated the significant hypoglycemic, hypolipidemic and antioxidant capacity of the EAE.

While the findings on the potential benefits of EAE derived from *M. splendens* are promising, further research is crucial to determine its safety and effectiveness as an adjunct herbal treatment for hyperglycemia, HTG and oxidative stress in individuals with DMI and DMII. It is essential to investigate the long-term effects, optimal dosages, and potential interactions with conventional diabetes medications, as well as the overall safety profile of this extract across diverse patient populations. Addressing the current limitations, including the lack of long-term toxicity data, variability in plant extract composition, seasonality, geographical distribution and the absence of human clinical trials, is essential to enhance the transparency and robustness of the findings. Moreover, future research should prioritize the investigation of the molecular pathways modulated by *M. splendens* to elucidate its therapeutic mechanisms and refine its potential applications in clinical practice.

Conflict of interests

The authors declare no competing interests.

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