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Chemical composition, antioxidant and gastrointestinal properties of *Sedum dendroideum* Moc & Sessé ex DC leaves tea infusion

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ABSTRACT

Ethnopharmacological relevance: Sedum dendroideum Moc & Sessé ex DC (Crassulaceae) is a medicinal plant employed in Mexican and Brasilian folk medicine as juice or infusion, as remedy for the treatment of different diseases, including gastric disorders.

Aim of the study: Although some studies carried out with *Sedum dendroideum* have demonstrated its gastroprotective effect, the purpose of this study was to elucidate the chemical constituents, antioxidant, cytotoxic and mechanisms underlying the gastrointestinal properties of *Sedum dendroideum* accordingly its traditional use, as fresh leaves tea infusion (SDI).

Materials and methods: Chemical constituents were analyzed using high performance liquid chromatography and mass spectrometry (HPLC-MS). Antioxidant and cytotoxicity were evaluated in *in vitro* assays. The efficacy of the SDI on macroscopic ulcer appearance, mucus and GSH maintenance on ethanol- and indomethacin-induced ulcer models, gastric acid secretion and gastrointestinal motility were investigated.

Results: Phytochemical analysis by HPLC–MS revealed the presence of different flavonol glycosides, containing myricetin and quercetin, along with the kaempferol as aglycones. *In vitro* pharmacological investigation of SDI demonstrated potent antioxidant activity in DPPH assay (IC50: 13.25 ± 3.37 μg/mL) and absence of cytotoxicity in Caco-2 cells by MTT method. Oral administration of SDI (ED_{50} of 191.00 ± 0.08 mg/kg) in rats promoted gastroprotection against ethanol or indomethacin in rats through reinforcement of gastric wall mucus, GSH content and nitric oxide release, without present antisecretory properties. The gastroprotective effect was maintained when SDI (19 mg/kg) was administrated by intraperitoneal route. Furthermore, SDI (150 mg/kg) unchanged the gastric emptying but increase small bowel transit in mice through cholinergic pathways.

Conclusions: Collectively, this study confirmed that *Sedum dendroideum* promotes gastroprotection through preventing of endogenous defense mechanisms, represented by mucus and GSH without changes gastric acid secretion. *Sedum dendroideum* tea infusion features a chemical profile that contributes to the antioxidant and gastric health-promoting effects, supporting the use in folk medicine for the treatment of gastrointestinal disorders.

1. Introduction

Sedum dendroideum Moc. & Sessé ex DC. (Crassulaceae) is a perennial and succulent plant widely used ornamentally. Originally from South Africa, this medicinal genus is used in Mexican culture for treatment of diabetes (Andrade-Cetto and Heinrich, 2005), eye

List of abbreviations: AA, Ascorbic acid; AlCl3, Aluminum chloride; API, Atmospheric pressure ionization; COX-1, Cyclooxygenase-1; COX-2, Cyclooxygenase-2; DPPH, 2,2′-diphenylpicryl hydrazyl; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); GSH, Glutathione; HPLC, High performance liquid chromatography; L-Arg, L-Arginina; L-NAME, N(ω)-nitro-L-arginine methyl ester; LC-MS, Liquid chromatography coupled mass spectrometry; MBM, Municipal Botanical Museum; MeOH, Metanol; MS, Mass spectrometry; NaCO3, Sodium carbonate; NaOH, Sodium hydroxide; NO, Nitric oxide; NSAIDs, Non-selective nonsteroidal anti-inflammatory drugs; PGE2, Prostaglandin E2; PPIs, Proton pump inhibitors; PQD, Pulsed quantum dissociation; ROS, Reactive oxygen species; SDI, Sedum dendroideum infusion; TIC, Total ion current

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inflammation and as contraceptive agent (Silva-Torres et al., 2003). In Brazil is commonly referred to as “bálsamo”, and where is popularly used as juice or infusion prepared by soaking the leaves in hot water for the treatment of gastric ulcers (Carlini et al., 1970; Rosas-Piñón et al., 2012).

Many bioactivities of different extracts and compounds isolated from *Sedum dendroideum* have been researched. With an incontestable pharmacological effect, previous studies showed that the *Sedum dendroideum* leaf juice exhibited antinociceptive and anti-inflammatory activities (De Melo et al., 2005). Moreover, isolated glycosides like kaempferol and kaempferitin promoted antinociceptive and anti-inflammatory effects in acetic acid-induced writhing and hypoglycemic activity streptozotocin-induced diabetes in mice (De Melo et al., 2009; Da Silva et al., 2014). The hydroethanolic extract of *Sedum dendroideum*, containing flavonoids, phenols, and tannins also showed gastroprotective action in rats (Carrasco et al., 2014). However, instead of consume alcoholic extracts with therapeutic purposes, a better insight into the gastroprotection promoted by *Sedum dendroideum* infusion (SDI) could harmonize and validate its popular use for the treatment of gastrointestinal disorders.

The adequate gastric function is essential for digestion and absorption of nutrients. However, disorders of the gastrointestinal tract as peptic ulcers are common, causing discomfort and abdominal pain. Peptic ulcers occurs due to exposition to acid and pepsin associated with the decrease of protective mechanisms of the mucosa, such impairment of mucus layer, antioxidant system and blood flux, which together to lifestyle habits, contributes to the gastric ulcer formation (Yandrapu and Sarosiek, 2015).

Considering the absence of valid ethnopharmacological studies of *Sedum dendroideum*, the aim of the present study was to characterize the chemical constituents and evaluate the antioxidant, antiulcer and prokinetic effects of an infusion prepared with leaves of *Sedum dendroideum* (SDI).

2. Materials and methods

2.1. Botanical material and infusion preparation

Sedum dendroideum were harvested in Campina Grande do Sul (25°19'05.3" S; 49°02'32.3" W, at 921 m above mean sea level), State of Parana (PR), South of Brazil. Dr. José Tadeu Weidlich Motta, plant taxonomist and curator of Municipal Botanical Museum (MBM) of Curitiba, PR, Brazil, identified the botanical material and a representative voucher specimen was deposited at the MBM herbarium (MBM-272917).

The infusion was prepared as previously published (de Oliveira et al., 2018). Briefly, 1.25 kg of dried leaves of *Sedum dendroideum* were submitted to extraction with boiling water (100 g/L) by infusion during 1 h. SDI was lyophilized in order to obtain a dry extract to determine the infusion concentration to perform *in vitro* and *in vivo* assays.

2.2. Phytochemical analysis

2.2.1. Liquid chromatography-mass spectrometry of SDI

The chromatography analysis was carried out in a high-performance liquid chromatography (HPLC, Agilent 1200) coupled to a mass spectrometry (MS) detector. The analysis were developed in a reversed-phase chromatography employing a BEH C18 column (50 × 2.1 mm with particle of 1.7 μm, from Waters), using ultra-pure water (Milli-Q) and acetonitrile (J.T. Baker) containing 0.1% of formic acid (v/v). A gradient of acetonitrile was developed, increasing from 5% to 20% (in 5 min), to 80% (in 10 min), returning to 5% (in 11 min), at 350 μL/min, with the column temperature at 60 °C and pressure did not exceed 4500 psi. Between analyses, the column was balanced for 3 min with the initial solvent. The sample was dissolved in MeOH-H_2O (1:1, v/v at 2 mg/mL) and 10 μL was injected.

The mass spectrometry analysis was developed with an electrospray ionization in a LTQ-Orbitrap XL (Thermo Scientific) at atmospheric pressure ionization (API), in the positive and negative ionization modes. The sample from LC was dried by a flow of nitrogen in the sheath gas and auxiliary gas (at 40 and 5 arbitrary units, respectively), with the source temperature of 300 °C. Positive and negative ions were obtained, however in the negative ionization mode, the compounds were better observed and fragmented. The negative ions were obtained using the spray at 3.2 kV, the tube lens at −200 V and the capillary at −46 V. The MS data was acquired in total ion current (TIC) and a data dependent event was used to fragment the base ion each peak, using a pulsed quantum dissociation activation (PQD) with normalized collision energy of 35.

2.2.2. Determination of the total phenolic and flavonoid contents in SDI

The total phenolic content was performed by spectroscopy using the colorimetric method described by Singleton et al. (1999). In 96-well plates, 20 μL of SDI (1 mg/mL) was mixed with 100 μL Folin-Ciocalteau phenol reagent. After 5 min, 80 μL of Na_2CO_3 (7.5%) was added and incubated for 120 min. Absorbance was measured at 760 nm using an automated microplate reader (Epoch™ Microplate Spectrophotometer-BioTek, Winooski, VT, USA). The total phenolic content was determined by interpolation of the samples absorbance with the standard curve of gallic acid (standard phenolic compound). The results were expressed in g gallic acid/100 g of sample.

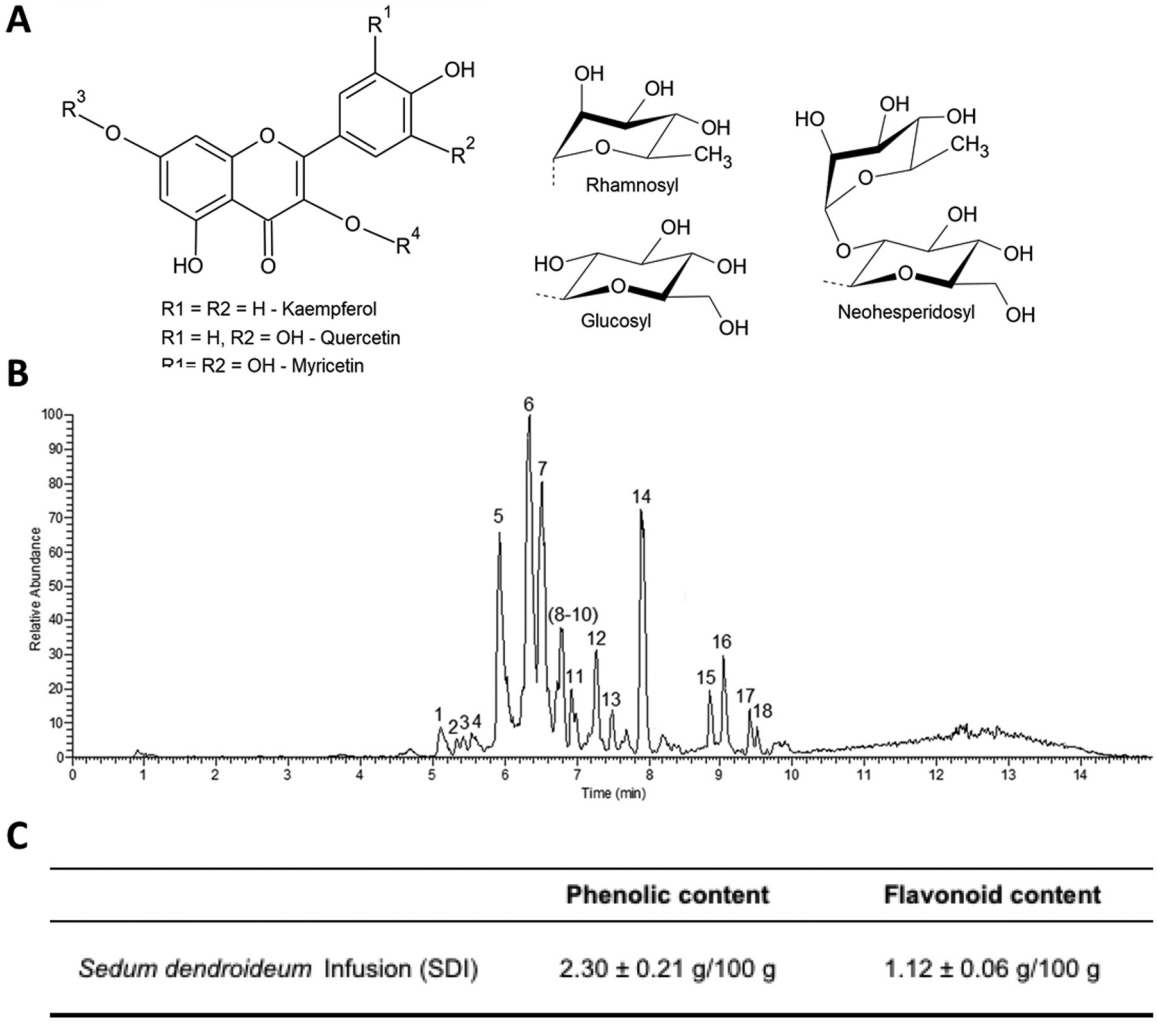
Total flavonoid content was determined using rutine as the standard flavonoid of (Quettier-Deleu et al., 2000). Briefly, 400 μL of SDI (1 mg/mL) were mixed with equal volume of $AlCl_3{\cdot}6H_2O$ methanol solution (2% w/v). After 10 min, the absorption of the standards was measured at 430 nm as previously described. The total flavonoid content was determined by interpolation of the samples absorbance with the standard curve of rutine. The results were expressed in g rutin/100 g of sample. All analyzes were performed in triplicate.

2.3. Determination of radical scavenging activity by DPPH in vitro method

Despite several plants present antioxidant activity, the DPPH (2,2′-diphenylpicryl hydrazyl) free-radical scavenging assay was used to estimate the antioxidant ability of SDI (Blois, 1958). Briefly, 225 μL of SDI (1, 10, 100 and 1000 μg/mL), ascorbic acid (AA, 50 μg/mL as positive control) or distilled water (as negative control) were mixed with 75 μL of methanolic DPPH solution (40 μg/mL) in a 96-well plate for 5 min. The absorbance was measured at 517 nm and values obtained were interpolated in a standard curve of DPPH (0–60 μM). Results were expressed as % of inhibition of DPPH.

2.4. Caco-2 cell culture and cytotoxicity assay

Human colon carcinoma cells line (Caco-2) were purchased from the Cell Bank of Rio de Janeiro, Brazil. Cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) and Ham´s-F12 (1:1), supplemented with 10% fetal bovine serum (FBS) and 100 IU/ mL penicillin/streptomycin. Cultures were maintained in humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Caco-2 cells were cultured in 96-well plates, at a density of 7×10^3 cells/well, and treated with increasing concentrations (10, 100, and 1000 μg/mL) of SDI diluted in FBS free medium. After 24 h of incubation, the solution was removed and 100 μL of MTT solution (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) (0.5 mg/mL) was added to each well and incubated for 3 h at 37 °C. Then, MTT solution was aspired and 100 μL of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals. Cell survival was assessed through absorbance determination at 570 nm. Medium alone was used as control and the cell viability was expressed as % of control cells.



	Phenolic content	Flavonoid content
Sedum dendroideum Infusion (SDI)	2.30 ± 0.21 g/100 g	1.12 ± 0.06 g/100 g

Fig. 1. Phytochemical analysis of SDI. Major flavonoids constituents in SDI (Panel A). HPLC–MS (high performance liquid chromatography coupled with mass spectrometer) profile of the SDI (Panel B). Quantification of total phenolic and flavonoid contents in SDI (Panel C).

Table 1
Phytochemical composition of SDI obtained by LC-MS/MS analysis.

Peak	Rt	MS^1(-)	MS^2(-)	Tentative identification
1	5.11	479.1	317.0, 316.0	Myricetin-hexoside
2	5.34	739.4	593.2, 430.1, 429.1, 285.0, 284.0	kaempferol 3-*O*-neohesperidoside 7-*O*-rhamnoside
3	5.43	609.3	463.1, 462.1, 317.0, 315.0	myricetin 3-*O*-rhamnoside 7-*O*-rhamnoside
4	5.53	609.3	463.1, 447.1, 446.1, 301.0, 300.0, 299.0	quercetin 3-*O*-glucoside 7-*O*-rhamnoside
5	5.93	593.2	447.1, 431.2, 430.1, 285.1, 284.1	kaempferol 3-*O*-glucoside 7-*O*-rhamnoside
6	6.34	577.3	431.1, 430.1, 285.0	kaempferol 3-*O*-rhamnoside 7-*O*-rhamnoside
7	6.51	737.3	675.3, 635.3, 593.3, 471.3, 429.1, 284.1	* kaempferol 3-*O*-rhamnoside 7-*O*-rhamnoside
8	6.69	577.3	431.1, 285.0	Kaempferitrin (isomer)
9	6.72	693.3	577.2, 431.1, 285.0	* kaempferitrin
10	6.77	591.3	529.1, 489.1, 447.2, 285.1, 284.0	* kaempferol glucoside
11	6.92	431.2	285.0, 284.0	kaempferol 3-*O*-rhamnoside
12	7.26	539.2	477.2, 437.2, 395.3, 377.1, 305.2, 275.0	n.i.
13	7.50	395.2	305.2, 275.1	n.i.
14	7.90	431.2	285.0, 284.0	kaempferol 7-*O*-rhamnoside
15	8.85	793.3	635.3, 593.2, 575.3, 471.3, 284.0	* kaempferol diglycoside
16	9.04	327.3	291.2, 229.2, 211.2	n.i.
17	9.40	329.3	293.2, 229.1, 211.1	n.i.
18	9.52	327.2	309.2, 291.2, 273.2, 201.2, 171.0	n.i.

n.i. = not identified.
* = structures containing a not identified group linked to flavonol-glycoside.

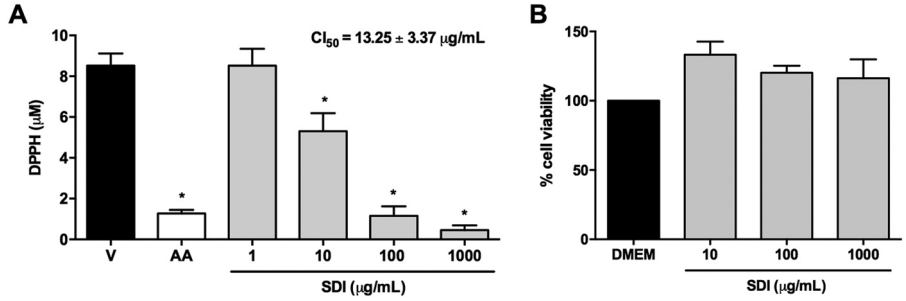


Fig. 2. Effects of SDI on DPPH radical scavenging activity (Panel A) and on Caco-2 cell viability (Panel B). Data on graph are representative of experiments performed at least three times in triplicate. **P < 0.05 when compared to vehicle group (V).

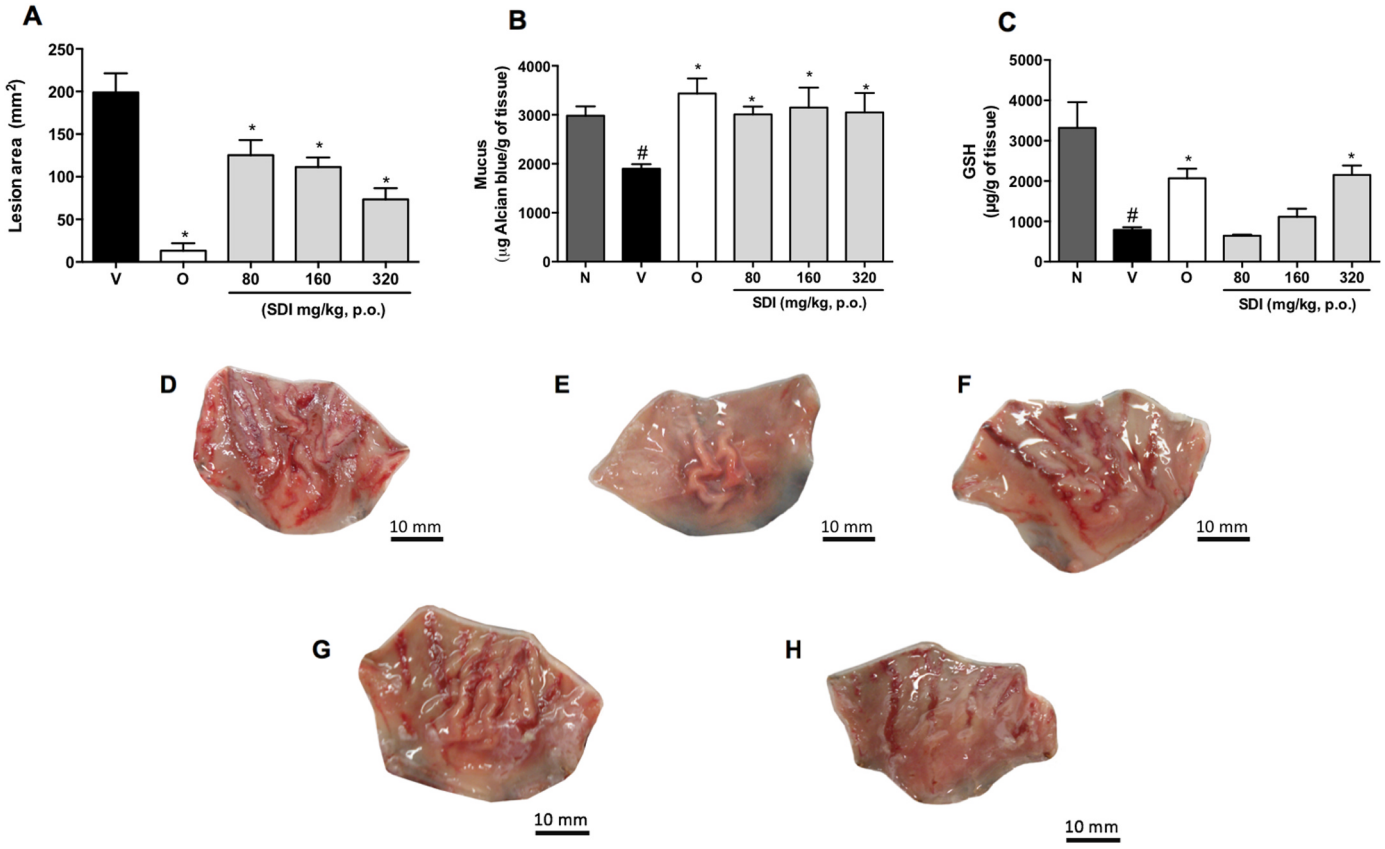


Fig. 3. Effect of oral pretreatment with SDI on gastric lesions induced by ethanol in rats. The panel A shows the gastric ulcer area (mm2); B mucus content (μg Alcian Blue/g of tissue) and C gluthatione levels (μg GSH/g of tissue). Panels D (Vehicle), E (Omeprazole), F (SDI: 80 mg/kg), G (SDI: 160 mg/kg) and H (SDI: 320 mg/kg) are representative of macroscopic photograph of stomachs. Results are expressed as mean ± S.E.M. *P < 0.05 when compared to vehicle group (V), #P < 0.05 when compared to naïve group (N).

2.5. Animals

Adult female Wistar rats (180–200 g) and *Swiss* mice (25–30 g) were provided by the Biotery of Federal University of Parana. Animals were housed under standard laboratory conditions: plastic cages (maximum of 5 rats and 20 mice per cage) with wood shaving bedding and free access to water and food, under a 12 h light/dark cycle and at controlled temperature (22 ± 2 °C). Fasting (16 h) was used prior all assays. The animals were kept in cages with raised, wide-mesh floors to prevent coprophagy with free access to water. All experimental procedures and animal handling were conducted in agreement with the "Guide for the Care and Use of Laboratory Animals" (8th edition, National Research Council, 2011) and approved by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA/BIO – UFPR 1010).

2.5.1. Dose conversion of SDI between human and animals

Popularly, *Sedum dendroideum* is consumed as infusion for treatment of gastric ulcers (prepared approximately with 5 leaves in 500 mL of hot water), three times a day. Using the total weight of 5 dried leaves (~0.6 g) and the daily consumption, the dose was extrapolated to the ingestion of 12 mg/kg/day for humans with a mean weight of 70 kg (Leite, 14 Benefícios do Bálsamo, 2017). Then, based on allometric scaling approach, the normalization of the human dose (12 mg/kg) was performed according to body surface area, providing the equivalent doses of 80, 160 and 320 mg/kg for rats and 150, 300 and 600 mg/kg for mice (Nair and Jacob, 2016). For reducing the number of rats in each experimental group, the median effective dose (ED_{50}) values were determined based on inhibition of gastric lesion induced by ethanol following SDI oral administration. Thus, the dose of 191 mg/kg was chosen to evaluate the following gastroprotective activity of SDI.

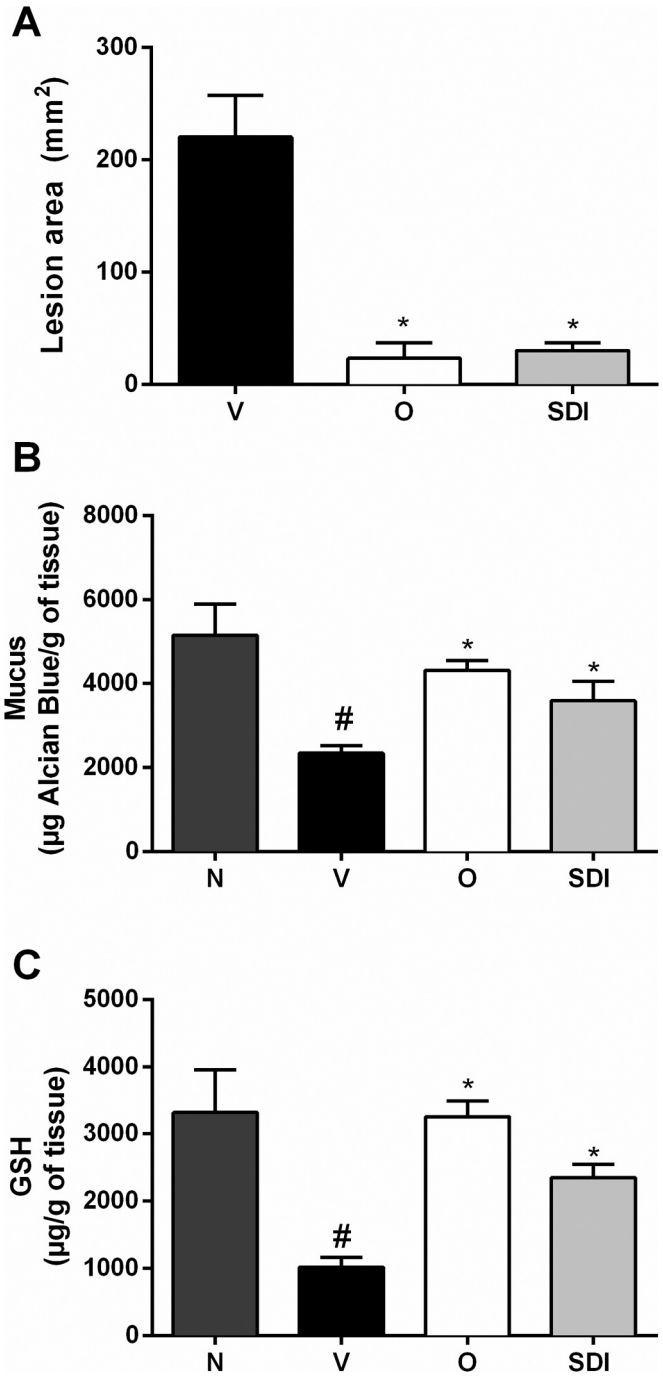


Fig. 4. Effect of intraperitoneal pretreatment with SDI on gastric lesions induced by ethanol in rats. The panel A shows the gastric ulcer area (mm2); B mucus content (µg Alcian Blue/g of tissue) and C gluthatione levels (µg GSH/g of tissue). The results are expressed as mean ± S.E.M. *P < 0.05 when compared to vehicle group (V), #P < 0.05 when compared to naive group (N).

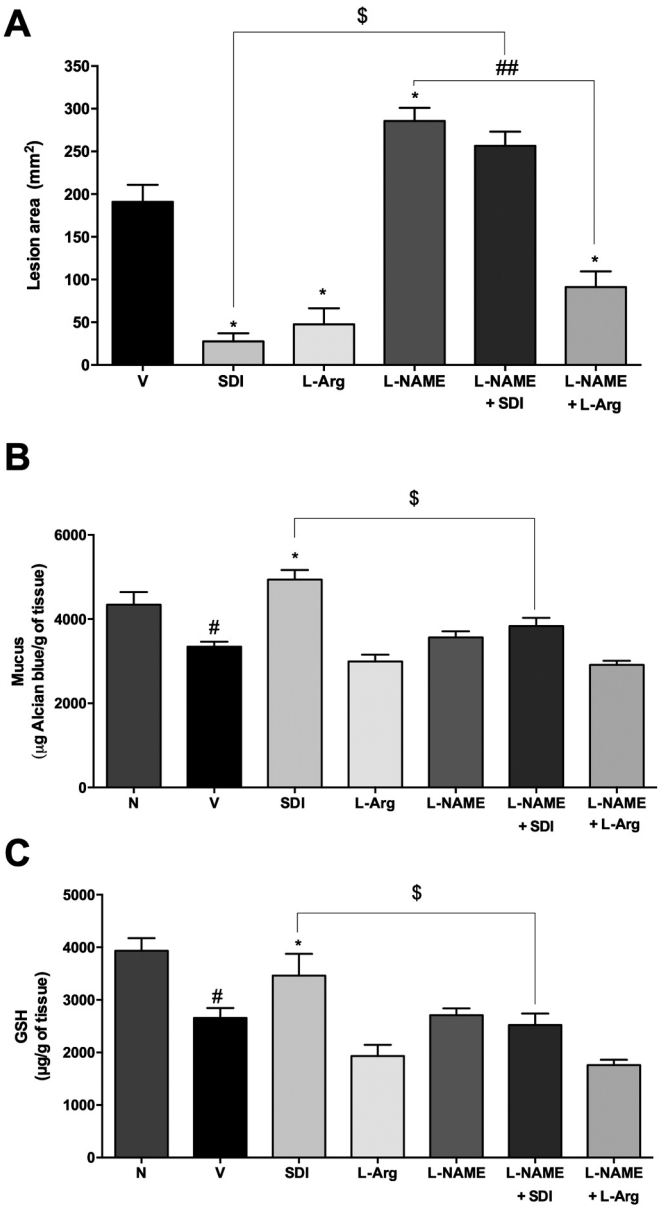


Fig. 5. Influence of L-NAME pretreatment on gastroprotection promoted by SDI on gastric lesions induced by ethanol in rats. The panel A shows the gastric ulcer area (mm2); B mucus content (µg Alcian Blue/g of tissue) and C gluthatione levels (µg GSH/g of tissue). The results are expressed as mean ± S.E.M. *P < 0.05 when compared to vehicle group (V). $P < 0.05 when compared to SDI group (SDI). ## P < 0.05 when compared to L-NAME group (L-NAME). #P < 0.05 when compared to naive group (N).

2.6. Pharmacological analysis

2.6.1. Acute gastric lesions induced by ethanol

The acute hemorrhagic lesions were induced by oral administration (v.o.) of ethanol (Robert et al., 1979). Animals were orally pretreated with water (Vehicle [V]: 1 mL/kg), omeprazole (O: 40 mg/kg) or SDI (80, 160 and 320 mg/kg, SDI ED_{50} 191 mg/kg, or SDI 19.1 mg/kg by intraperitoneal route, i.p.). Sixty or 30 min after oral or intraperitoneal treatments, respectively, all animals received ethanol P.A. (1 mL/rat) and then, animals were euthanized 1 h later by thiopental overdose (100 mg/kg, i.p.). To analyze the gastric lesions, the stomachs were excised, opened along the smaller curvature and photographed to provide visual evidence of hemorrhagic ulcers. All ulcer wound was measured by computerized planimetry using the program Image Tool® 3.0, and the lesion area was expressed in mm^2.

2.6.2. Involvement of endogenous nitric oxide in the gastroprotective effect of SDI

To investigate the involvement of endogenous nitric oxide (NO) in the gastroprotective effect of SDI, rats were pretreated with Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME, 20 mg/kg, i.p.). Following 30 min, the rats received a single oral dose of L-Arginine (L-Arg: 200 mg/kg) or SDI (ED_{50} 191 mg/kg), 1 h before oral administration of ethanol

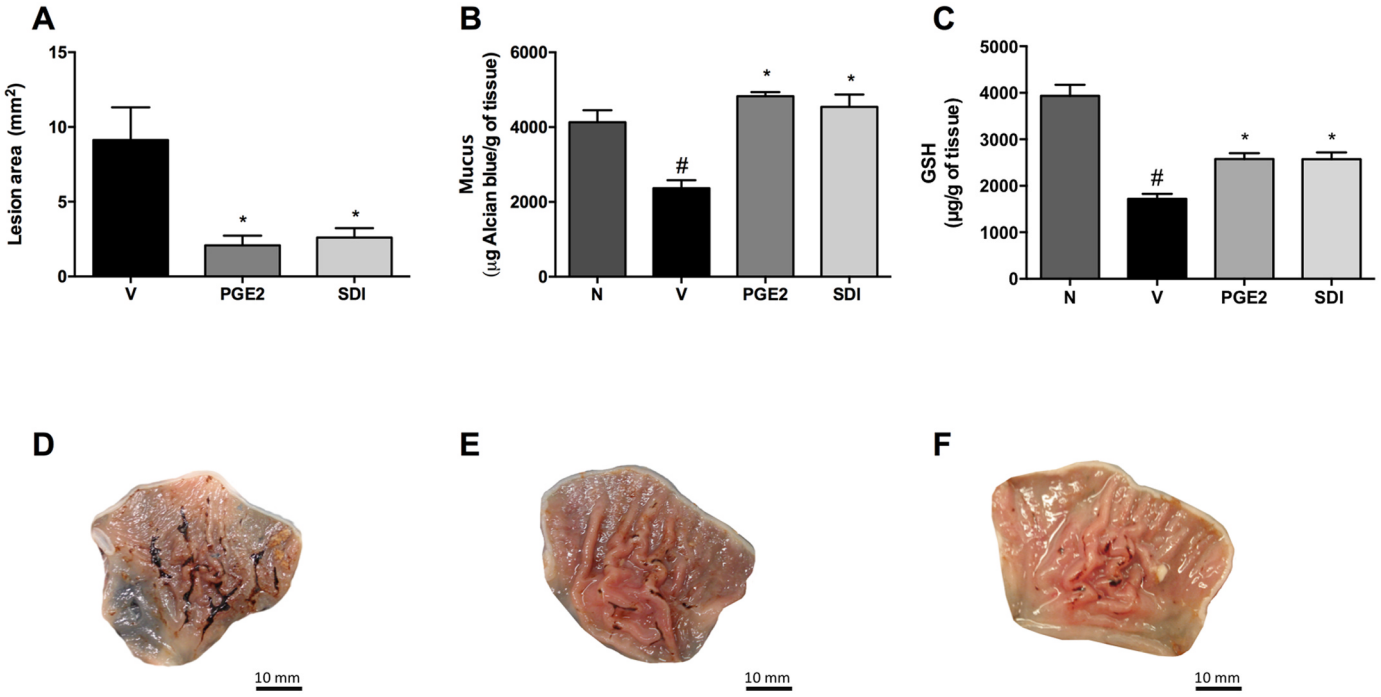


Fig. 6. Effect of oral treatment with SDI on gastric lesions induced by indomethacin in rats. The panel A shows the gastric ulcer area (mm2); B mucus content (µg Alcian Blue/g of tissue) and C gluthatione levels (µg GSH/g of tissue). Panels D (Vehicle), E (Prostaglandin E2) and F (SDI: 191 mg/kg) are representative of macroscopic photograph of stomachs. The results are expressed as mean ± S.E.M. *P < 0.05 when compared to vehicle group (V). #P < 0.05 when compared to naive group (N).

P.A. (1 mL/rat). Then, animals were euthanized 1 h later, and gastric lesions were analyzed as described above.

2.6.3. Acute lesions induced by indomethacin

The rats were pretreated by oral route with water (vehicle [V]: 1 mL/kg), Prostaglandin E_2 (PGE_2: 20 µg/kg) and SDI (ED_{50} 191 mg/kg). One h after the treatments, all animals received a single oral dose of indomethacin (100 mg/kg) and then, animals were euthanized 6 h as previously described. The same procedure was employed to analyze the indomethacin-induced gastric lesions.

2.6.4. Determination of gastric wall mucus

The evaluation of gastric wall mucus content was performed in stomachs from ethanol- and indomethacin-induced lesions, according to the reported method (Corne et al., 1974). First, the glandular segment of stomach was complexed with a dye solution of 0.1% Alcian Blue during 2 h. After, the tissue was washed with 250 mM sucrose twice for 15 and 45 min respectively, and then the complex mucus-dye was extracted adding 500 mM magnesium chloride and stirred intermittently for 2 h. The solution extracted was mixed with the same ether volume and centrifuged for 10 min at 3600 rpm. The aqueous layer was separated to measure the absorbance at 580 nm and the results were expressed in µg Alcian blue/g of glandular tissue.

2.6.5. Determination of gastric glutathione levels

The samples of stomach from ethanol- and indomethacin-induced lesions were homogenized with cold 200 mM potassium phosphate buffer (pH 6.5) in a volume equal to 3 times the weight of fresh gastric tissue to determinate glutathione (GSH) levels as previously described (Sedlak and Lindsay, 1968). Aliquots of samples were mixed and vigorously shaken with 12.5% trichloroacetic acid (ATC) before being centrifuged at 3000 rpm for 15 min at 4 °C. The supernatant of the samples, 400 mM TRIS-HCl buffer (pH 8.5) and 10 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were added in a 96-well plate to perform the colorimetric reaction. Then, the absorbance was read at 412 nm. The values obtained were interpolated in a standard curve of GSH and the results were expressed as µg GSH/g of tissue.

2.6.6. Induction of hypersecretion by pylorus ligation in rats

The gastric secretion was evaluated as previously described (Shay, 1945). The rats were anesthetized with halothane, and after laparotomy, the stomach was exposed and the pylorus was tied. The animals were treated through intraduodenal route with vehicle (V: water 1 mL/kg, i.d.), SDI (ED_{50} 191 mg/kg, i.d.) or by oral route with the positive control omeprazole (O: 40 mg/kg, v.o.), 1 h before the pylorus ligation. After 4 h, animals were euthanized, the stomach was pinched, and gastric juice collected and centrifuged to measurements of the secreted volume, pH and total acidity.

2.6.7. Evaluation of prokinetic properties through gastric emptying and intestinal motility

For determinate the gastric emptying and small intestinal transit, mice were pretreated by oral route with vehicle (V: Water 1 mL/kg) and SDI (150, 300 and 600 mg/kg). After 1 h, all animals received 1.5% phenol red marker (0.5 mL/mice) and then, animals were euthanized 20 min later. The abdominal cavity was opened and the stomach and the small intestine until the cecum were immediately removed for evaluation of phenol red marker position (Suchitra, 2003).

To measure the gastric emptying, the stomach and its contents were homogenized with water and centrifuged for 15 min at 1500 rpm. Then, 150 µL of the supernatant plus 150 µL of 0.1 N NaOH were added in a 96-well plate for colorimetric reaction and reading at 560 nm. Gastric emptying was measured as the amount of marker that remained in the stomach at the end of the experiment, and the results were expressed as %.

In another set of experiments, to address some of the mechanisms by which SDI modulates small intestinal transit, mice were pretreated subcutaneously with atropine (5 mg/kg, s.c.) or orally with loperamide (5 mg/kg, v.o.), 30 or 60 min, respectively, before the treatment with SDI (150 mg/kg, v.o.). Following 1 h, all animals received 1.5% phenol

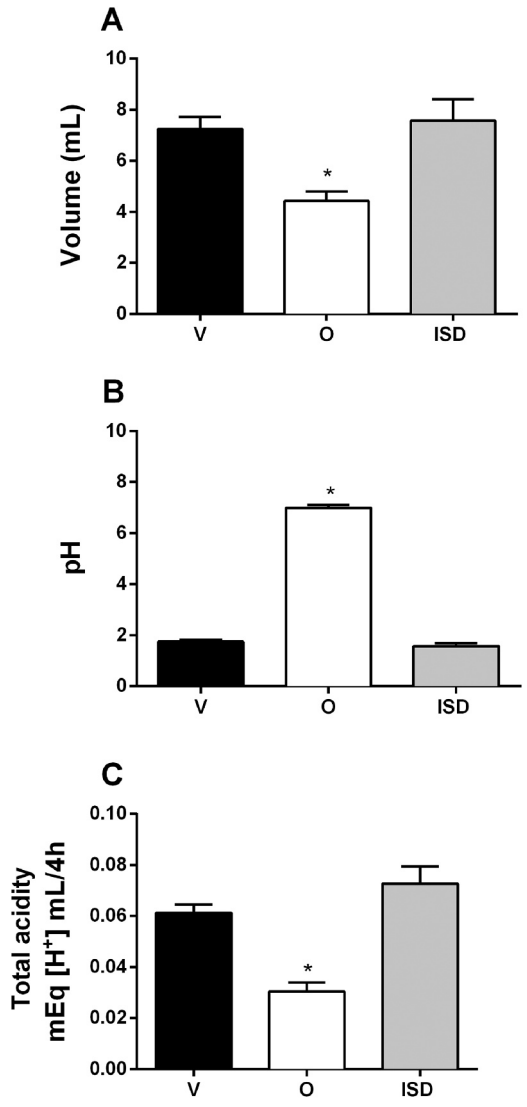


Fig. 7. Effect of oral treatment with SDI on the gastric secretion by pylorus ligation in rats. The panel A shows the volume (mL); B pH and C total acidity (mEq [H+] mL/4 h). The results are expressed as mean ± S.E.M. *P < 0.05 when compared to vehicle group (V).

red marker (0.5 mL/mice). Then, the small intestine was dissected from the pylorus to the ileocaecal junction and the intestinal transit was measured considering the total length of the small intestine and the distance covered by phenol red solution. The results were expressed as %.

2.7. Statistical analysis

Data were expressed as mean ± standard error of the means (S.E.M.). Statistical differences between experimental groups (n = 6–9 animals per group) were analyzed with one-way ANOVA followed by Bonferroni's multi-comparison post-hoc test. The ED_{50} values (effective dose capable of inhibiting the gastric lesions formation by 50% relative to the control group values) or IC_{50} values (inhibitory concentration required to obtain a 50% antioxidant effect in DPPH assay) were determined by nonlinear regression analysis and reported as geometric mean. All analyzes were performed using the GraphPad Prism® version 6.0 (GraphPad Software, San Diego, USA). Differences were significant when $P \leq 0.05$.

3. Results and discussion

3.1. Phytochemical investigation

In previous investigation (De Melo et al., 2005, 2009; Da Silva et al., 2014), some flavonol glycosides from *Sedum dendroideum* were identified, containing mainly kaempferol as aglycone. De Melo et al. (2009) founded the kaempferol attached mainly by rhamnose (Rha), with glucose (Glc) at lower abundance, linked in the positions 3 and/or 7.

In our current analysis, using LC-MS in the negative ionization to produce deprotonated ions $[M-H]^-$, we have found different flavonol glycosides, containing myricetin and quercetin, along with the kaempferol as aglycones (Fig. 1A). In SDI, the first peak (**1**) was observed at *m/z* 479.2 with fragments at *m/z* 317.0 and 316.0, being consistent with myricetin-hexoside. The fragments observed are characteristics of myricetin, produced two types of linkage breakdown, the heterolytic cleavage, yielding the regular ion (*i.e. m/z* 317.0) and, by a homolytic cleavage, a radical ion was produced (*i.e. m/z* 316.0$^{\bullet}$). Similarly, the other aglycones also produced these two ions from aglycones, being at *m/z* 285/284 for kaempferol and *m/z* 301/300 for quercetin. However, since these flavonols can be linked to glycans at two positions, each linkage could undergoes to a hetero- and/or hemolytic cleavage, yielding to different ions. The superscript symbol ($^{\bullet}$) will be used to indicate a radical ion.

The peak **2**, at *m/z* 739.3 with main fragments at *m/z* 593.3 (-Rha), 447.2 (-Rha_2), 430.1$^{\bullet}$ (-Rha, -Glc) and 284.0$^{\bullet}$ (from kaempferol), being consistent with kaempferol 3-*O*-neohesperidoside 7-*O*-rhamnoside (De Melo et al., 2005, 2009; Da Silva et al., 2014). The peak **3**, at *m/z* 609.3 and fragments at *m/z* 462.1$^{\bullet}$, 317.0 and 315.1$^{\bullet\bullet}$ was consistent with myricetin 3-*O*-rhamnoside 7-*O*-rhamnoside. The ion at *m/z* 315.1$^{\bullet\bullet}$ was assigned as a product from a double homolytic cleavage, indicative of two glycosylation sites. The peak **4**, at *m/z* 609.3 had different fragments from peak **3**, with those at *m/z* 463.2, 447.2/446.3$^{\bullet}$ and the aglycone ions at *m/z* 301.1, 300.1$^{\bullet}$ and 299.0$^{\bullet\bullet}$, suggesting a quercetin 3-*O*-glucoside 7-*O*-rhamnoside.

The abundant peak **5**, was found at *m/z* 593.3 and fragments at *m/z* 447.2, 431.2/430.2$^{\bullet}$ and the aglycone at *m/z* 285.0 (main), 284.0$^{\bullet}$ and 283.0$^{\bullet\bullet}$ (lower). This compound is consistent with the kaempferol 3-*O*-glucoside 7-*O*-rhamnoside. The most abundant peak (**6**) was consistent with kaempferitrin (kaempferol 3-*O*-rhamnoside 7-*O*-rhamnoside), observed at *m/z* 577.2 and fragments at *m/z* 431.1/430.1$^{\bullet}$ and 285.0/283.1$^{\bullet\bullet}$. Another abundant peak (**7**) gave the ion at *m/z* 737.3 and main fragments at *m/z* 675.3, 635.3, 593.2, 429.2 and 284.0$^{\bullet}$. Although the fragments at *m/z* 284.0$^{\bullet}$ and 593.3 could suggest a kaempferol-glucorhamnoside, the other fragments could not be property identified. The fragment at *m/z* 635.3 is consistent with an acetyl group linked to glycoside, however the fragment at *m/z* 675.3 was not assigned. Porter et al. (2012) have characterized some flavonol glycosides with a substituent of 3-hydroxy-3-mehtylglucaric acid. Although the structure from Porter and coworkers had similar ion at *m/z* 737, with our fragmentation profile we could not confirm the identity of peak **7**.

The peak **8**, observed at *m/z* 577.3 and fragments at *m/z* 431.2 and 285.1 was consistent with an isomer of kaempferitrin. The peak **9** at *m/z* 693.3 and fragments at *m/z* 577.3, 431.1/430.2$^{\bullet}$, 285.0 was consistent with kaempferitrin containing an unknown substituent. The peak **10** appeared at *m/z* 591.3, and *m/z* 529.2, 489.2, 447.2 and 285.0/284.0$^{\bullet}$. The fragments at *m/z* 285.0/284.0$^{\bullet}$ and 447.2 are consistent with a kaempferol glucoside, the ion at *m/z* 489.2 suggest an acetylation but the ion at *m/z* 529.2 was not identified. This compound has a similar substituent of peak **7**.

The peak **11** at *m/z* 431.2 and 285.0/284.0$^{\bullet}$ is consistent with a kaempferol 3-*O*-rhamnoside, considering its lower abundance in relation to the isomer (peak **14**). The peak **12**, at *m/z* 539.4, gave rise to fragmentation profile different from common flavonoid-glycosides. However, the more intense product-ions at *m/z* 437.1 and 275.0 (neutral loss 162 amu) are consistent with glycoside. The peak **13** was

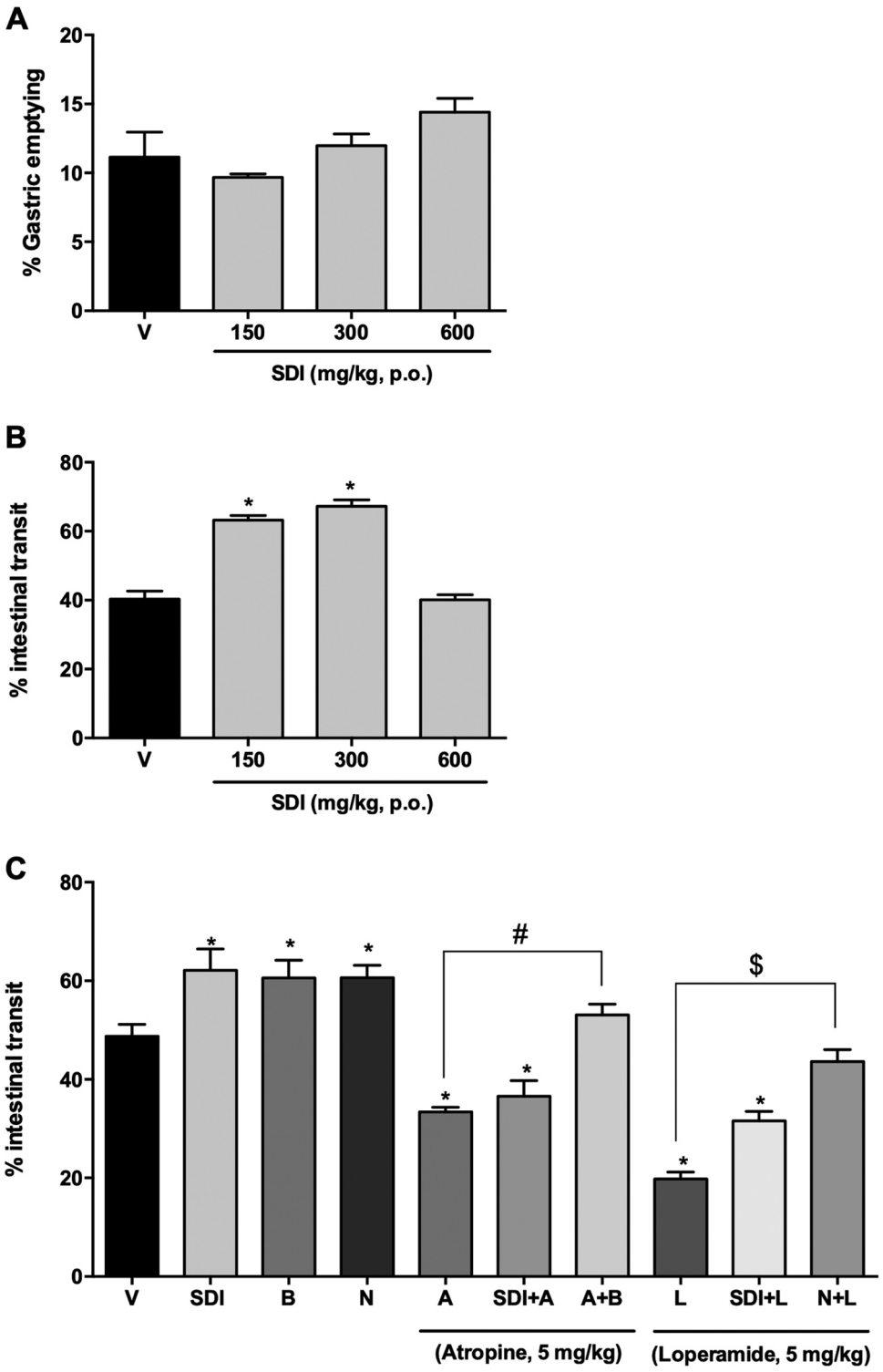


Fig. 8. Effect of oral treatment with SDI on the gastric emptying and intestinal motility in mice. The panel A shows the % gastric emptying, B % of intestinal motility and C the mechanism of action underlies SDI effect on % of intestinal motility. The results are expressed as mean ± S.E.M. *P < 0.05 when compared to vehicle group (V). #P < 0.05 when compared to SDI group (SDI). $P < 0.05 when compared to Loperamide group (L).

not identified, observed at *m/z* 395.2, it had similar ions to peak **12**, with the product-ion at *m/z* 275.0 being the highest. The peak **14**, at *m/z* 431 and fragments at *m/z* 285/284.0˙ is consistent with kaempferol 7-*O*-rhamnoside, considering that kaempferol 7-*O*-glycosides are the main compounds in *Sedum dendroideum* (De Melo et al., 2005, 2009; Da Silva et al., 2014). The peak **15**, was observed at *m/z* 793.4, with prominent fragments at *m/z* 635.2, 593.2 and 285.0/284.0˙. The product-ions at *m/z* 285.0/284.0˙ are consistent with kaempferol and that at *m/z* 593.2, with a diglycoside (*e.g.* glucose + rhamnose). Similarly to the peak **7**, the fragment at *m/z* 635.2 is consistent with an acetyl group attached to kaempferol diglycoside, as previously observed (Abdel-Hameed et al., 2013), however, an unknown group seems to be attached to this structure. The last peaks found in the chromatogram (**16**, **17**, **18**) were not identified (Fig. 1B). The results are summarized in the Table 1.

The quantification of total phenolic compounds and flavonoid contents confirmed the presence of these antioxidant compounds in the *Sedum dendroideum* tea infusion (2.30 ± 0.21 and 1.12 ± 0.06 g/100 g SDI, respectively), which suggested that SDI contains considerable amount of important secondary metabolites (Fig. 1C). Particularly, phenolic compounds are among the major phytochemical compounds responsible for the antioxidant activity of plants and dietary supplements (Guldiken et al., 2018).

In fact, we observed that SDI had direct DPPH radical-scavenging ability. The scavenging effect of SDI on DPPH radicals was found to increase in concentration dependent manner (10, 100 and 1000 μg/mL), decreasing the DPPH free radicals in 37.74%, 86.45% and 94.69% respectively, when compared to vehicle group (V: 8.52 ± 0.584 μM) (SDI IC_{50}: 13.25 ± 3.37 μg/mL). In addition, the ascorbic acid or vitamin C used as a positive control, an essential cofactor for several enzymes and powerful antioxidant, decreased the DPPH free radicals in 85.08% when compared to the vehicle group (Fig. 2A). These results were expected because of the high percentage of phenolic components and flavonoids in SDI, and reinforce the notion that in tea infusions, these antioxidants components exert a positively correlation in terms of concentration (IC_{50}) (Fotakis et al., 2016). As shown in Fig. 2B, the results from MTT assay demonstrate that 24 h incubation of Caco-2 cells with SDI 10–1000 μg/mL did not show changed cell viability when compared to control medium, demonstrating that SDI did not display cytotoxic effects.

3.2. Pharmacological investigation

Our results demonstrated that *Sedum dendroideum* leaves infusion prepared accordingly the traditional use of the plant exert beneficial effects on gastrointestinal tract as an antiulcer and prokinetic agent.

It is well know that ethanol is an exogenous aggressive factor for ulcer development, causing directly gastric damage, leading to the formation of acute hemorrhagic lesions, as can be seen in the Fig. 3D. Moreover, the destruction of protective factors such as mucus barrier and the depletion of GSH, also contribute to the development of the ethanol induced-ulcer, favoring the generation of reactive oxygen species (ROS), free radicals and lipoperoxidation, culminating in oxidative stress and further gastric injury (Yang et al., 2017). SDI administered by oral route at doses of 80, 160 and 320 mg/kg significantly reduced the ethanol-induced gastric lesions in 37.04%, 44.06% and 63.04% respectively when compared to the vehicle control group (V: 198.93 ± 22.41 mm^2), with an ED_{50} of 191.00 ± 0.08 mg/kg. Omeprazole (40 mg/kg,) also prevented the ulcer formation in 93.35% (Fig. 3A, D–H). The mucus-bicarbonate barrier constitutes the first line of defense of the gastric mucosa against acid and pepsin, maintaining luminal pH between 7.0. Accordingly, SDI (80, 160 and 320 mg/kg) preserved significantly the gastric mucus levels when compared to the vehicle control group in 36.84%, 39.67% and 37.71%, respectively (V: 1899.15 ± 121.68 μg of Alcian blue/g of tissue), while omeprazole preserved mucus in 44.69% (Fig. 3B). Moreover, the decrease of GSH levels, which is an essential endogenous antioxidant, greatly impairs its important action to limit the toxicity of ethanol (Brown et al., 2004). As illustrated in Fig. 3C, SDI (320 mg/kg) and omeprazole replenish the GSH levels in 63.35% and 61.88%, respectively, when compared to the vehicle control group (V: 788.57 ± 69.02 μg GSH/g of tissue).

In a subsequent experiment, rats were pretreated with SDI at 19.1 mg/kg, a 10-fold lower dose (in relation to the ED_{50} obtained with the oral administration of SDI), by intraperitoneal route to discard a possible physical barrier formation on the gastric mucosa prior to the ethanol administration. It is important to mention that only the ED_{50} was employed, aiming to reduce the number of experimental animals, in agreement to the 3Rs principles. Interestingly, the gastroprotective effect promoted by SDI remained. The intraperitoneal administration of SDI (19.1 mg/kg) significantly reduced the gastric lesions induced by ethanol in 86.25% and preserved the mucus and GSH depletion in 34.74% and 56.95%, respectively (Fig. 4). These observations reinforce the notion that the gastroprotection promoted by oral administration of SDI does not occur only by a physical barrier formation, as occurs with sucralfate treatment, in which the main mechanism of action is attributable to the formation of a protective barrier over the eroded mucosa (Sulochana et al., 2016).

Considering that gastric mucosa is continuously exposed to endogenous and exogenous aggressive agents, it is remarkable that the gastric microcirculation has also been intimately implicated in the maintenance of mucosal integrity. In this regard, mucosal blood perfusion is physiologically dependent of NO, an important vasodilator that also mediates the stimulation of gastric mucus secretion, increasing both endogenous protective factors (Kato et al., 1998). In the ethanol-induced ulcer model, the pretreatment of animals with L-NAME, a NO synthase inhibitor, completely abolished the gastroprotective effect of both SDI (191 mg/kg) and L-arginine, a precursor of endogenous NO synthesis (200 mg/kg), including the mucus and GSH depletion (Fig. 5). Again, under conditions of oxidative stress observed in ulcerogenic process, dietary flavonoids founded in SDI may increase NO production and protect its inactivation. These results could explain that endogenous NO is involved in the gastroprotection promoted by SDI.

Epidemiologic studies indicate that patients treated with non-selective nonsteroidal anti-inflammatory drugs (NSAIDs) have a higher risk of develop gastric ulcers (Drini, 2017). Indomethacin is a non-selective NSAID, prescribed for a variety of inflammatory pathologies, including arthritis. Despite its therapeutic effects, NSAIDs can cause bleeding, ulceration and stomach perforation, as consequence of cyclooxygenase isoenzymes COX-1 and COX-2 inhibition that are responsible for the production of gastroprotective prostaglandins (PGE_2 and PGI_2) (Wallace, 2008). The results depicted in Fig. 6A reveals that the SDI (191 mg/kg, p.o.) and PGE_2 (20 μg/kg) reduced significantly the indomethacin-induced gastric ulcers in 71.51% and 77.22%, respectively, when compared with the vehicle group (V: 9.13 ± 2.17 mm^2, SDI: 2.60 ± 0.63 mm^2). Again, the gastroprotective effect of SDI also seems to be related to the maintenance of protective factors, since SDI inhibited the depletion of mucus and glutathione levels in 47.86% and 33.17% respectively, when compared to the vehicle group (V: Mucus: 2369.41 ± 214.40 μg alcian blue/g of tissue; GSH: 1719.29 ± 109.53 μg GSH/g of tissue) (Fig. 5B-C). In sharp contrast, Baracho et al. (2014) observed that the aqueous extract of *Sedum dendroideum* leaves worsening the gastric ulcers induced by indomethacin, suggesting that bálsamo *per se* could induces gastritis. However, it is important to mention that the authors were not able to determine the main components neither stablish the doses of aqueous extract employed in this study.

Moreover, it was addressed if SDI could affect the gastric acid secretion. Here, we observed that SDI administration in animals with gastric hypersecretion induced by pylorus ligature did not alter volume, pH or total acidity of gastric secretion when compared with vehicle control group (V: volume: 7.257 ± 0.461 mL; pH: 1.742 ± 0.07; Total acidity: 0.0612 ± 0.0032 mEq[H^+]/mL) (Fig. 7). As expected, the positive control of the test, omeprazole decreased the secreted volume, pH and total acidity, probably due its mechanism of action, represented by the inhibition of proton pumps in the parietal cell ducts (O: Volume: 4.429 ± 0.366 mL; pH: 6.99 ± 0.119; Total acidity: 0.0304 ± 0.0034 mEq[H^+]/mL). Among medicinal plants popularly used as infusion to treat gastric complains, we found that the infusion of *Curatella Americana*, which is rich in flavonol glycosides such as quercetin, does not exert gastroprotection by antisecretory mechanisms (El-Azizi et al., 1980; Hiruma-Lima et al., 2009). In addition, quercetin *per se* showed antioxidant effects and protected gastric mucosa against indomethacin-induced ulcers (Alkushi and Elsawy, 2017).

Moreover, our results are partially in accordance with previous studies obtained with the hydroethanolic extract of *Sedum dendroideum* (Carrasco et al., 2014). The authors observed no signs of toxicity and a significantly inhibition of gastric ulcers which was accompanied by the

prevention of gastric mucus levels in the ethanol- and indomethacin-induced injury, with lower doses of hydroethanolic extract (25, 50 and 100 mg/kg) when comparing to the SDI (ED_{50}: 191 mg/kg). On the other hand, the antiulcer effect of hydroethanolic extract of *Sedum dendroideum* is not dependent of NO and was attributed to the antisecretory activity.

Interestingly, using the hydroethanol as solvent, Carrasco and coworkers (2014) found the presence of flavonoids (quercetin, rutin, and luteolin), phenols, and tannins, whereas SDI prepared as infusion, using only water as solvent yield mostly sugars, phenols and flavonol glycosides (myricetin, quercetin and kaempferol), proving that different solvents yield different composition of secondary metabolites. Additionally, the phytochemical constituents of SDI may also play an essential role in the observed results. Interestingly, flavonoids, kaempferol and quercetin, the main constituents found in SDI infusion, have been previously studied in several ulcer models as possible gastrointestinal protective agents (Li et al., 2018; Kahraman et al., 2003). Moreover, we demonstrated that a polysaccharide fraction, constituted by a homogalacturonan and a homogalacturonan branched by side chains of arabinans and type II arabinogalactans, obtained and isolated from SDI also promotes gastroprotection (de Oliveira et al., 2018). This pectic polysaccharide reduced ethanol-induced gastric ulcers in rats through preservation of mucus barrier and GSH levels in gastric tissue. In this sense, in addition to the secondary metabolites, primary metabolites as polysaccharides are also involved in the gastroprotective effects of SDI.

Thus, it is evident that SDI presents mixtures of active compounds that could act synergistically to exert the antioxidant and antiulcer effects observed in our study. Therefore, it seems unlikely that such a broad spectrum of mucosal protection as that exerted by SDI depends on a unique mechanism of action, but surely is not associated with antisecretory effects, different from the results of the hydroethanolic extract, that inhibited the gastric secretion and the acidity of the stomach (Carrasco et al., 2014). Thus, SDI could offer a safety treatment option when compared with reference drugs, such as omeprazole. Recent observational studies have associated the long-term use of PPIs with some unwanted effects, like nutritional deficiency related to malabsorption of nutrients, risk of bone fracture and risk of *Clostridium difficile* enteric infections, all related to the modification of stomach and intestinal pH (Nehra et al., 2018).

The pathogenesis of peptic ulcer disease involves multiple causes, for instance, the exogenous factors which have already been considered, genetic factors and endogenous factors, such as pathophysiological disorders, including abnormal motility and gastric empty (Quigley, 2017). SDI did not display gastric emptying effects in mice when compared to the vehicle (V: 11.13 ± 1.82%) (Fig. 8A). However, it is well known that prokinetics drugs are used to relief the gastric symptoms. Therefore, natural products, like SDI, may be an interesting alternative, once the pretreatment of mice increased the intestinal motility in 56.99% and 66.90% at doses of 150 and 300 mg/kg, respectively, when compared to the vehicle group (V: 40.27 ± 2.37%) (Fig. 8B). Regarding the mechanisms underlying the increase of the small intestinal transit promoted by SDI, it was obviously that both atropine, a muscarinic receptor antagonist, and loperamide, a μ-opioid receptor agonist, inhibited intestinal motility. However, the prokinetic effect of SDI 150 mg/kg was blunted in animals treated with atropine and loperamide, decreasing in 30.88% and 60.76% the intestinal transit, respectively, when compared to vehicle group (V: 46.84 ± 3.17%) (Fig. 8C). Altogether, our data indicates that SDI could increases peristalsis through cholinergic pathways signaling, once atropine directly blockade the cholinergic transmission and loperamide through the activation of μ-opioid receptors has the same effect, decreasing the peristalsis and consequently the intestinal motility, reversing the prokinetic effect of SDI. Considering the constituents characterized in SDI, is reasonable suggest that both polyphenolic compounds and pectic polysaccharides could improve the digestive health (de Oliveira et al., 2018; Ammar et al., 2018; Wang et al., 2018).

4. Conclusion

Collectively, our results show that *Sedum dendroideum* tea infusion prepared in accordance to the ethnopharmacological use contains several phenolic components with antioxidant properties, specially flavonols as quercetin, myricetin and kaempferol, and their glycosides, all concentrated following aqueous hot extraction. The phytochemical compounds found in SDI promotes gastroprotection against ethanol- and indomethacin-induced ulcers, through the reinforce of mucosal integrity, represented by maintenance of gastric mucus and GSH levels and the NO-mediated blood flow, without changes in acid secretion and no apparent signs of toxicity in colonic cells *in vitro*. This detailed mechanistic study provided a scientific basis for the popular use of *Sedum dendroideum*, reinforcing the unquestionable gastroprotective effect of an edible plant with a promising bioactivity to prevent gastric complaints.

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Declarations of interest

The authors declare that they have no conflict of interest.

Author contributions

M.F. Werner, B.B. da Luz and D. Maria-Ferreira conceived and designed the study. T.R. Cipriani, L.M. de Souza and A.F. Oliveira carried out the infuse preparation and the phytochemical investigation. B.B. da Luz, D. Maria-Ferreira and J.L. Dallazen performed the experiments and data analysis. M.F. Werner and B.B. da Luz wrote and reviewed the manuscript.

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Effectiveness of the polyphenols-rich *Sedum dendroideum* infusion on gastric ulcer healing in rats: Roles of protective endogenous factors and antioxidant and anti-inflammatory mechanisms

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ABSTRACT

Ethnopharmacological relevance: Peptic ulcer is an inflammatory disease that therapeutic options are mainly focused in antisecretory drugs. **S***edum dendroideum Moc & Sessé ex DC (Crassulaceae)* is employed in folk medicine for the treatment of gastric ulcers. Recently, our group demonstrated that *Sedum dendroideum* infusion (SDI) is rich in polyphenols (flavonol glycosides, myricetin, quercetin and kaempferol) and promoted gastroprotection against acute ulcer models, without changes gastric acid secretion.

Aim of the study: Here, we follow the investigation of the healing effects of SDI (ED_{50} = 191 mg/kg) in the chronic gastric ulcer model induced by 80% acetic acid in rats, elucidating underlying mechanisms.

Material and methods: Rats were orally treated with vehicle (water, 1 mL/kg), SDI (191 mg/kg), omeprazole (40 mg/kg) or sucralfate (100 mg/kg) twice daily for 5 days after ulcer induction. Following treatments, toxicological effects, macroscopic ulcer appearance, microscopic histological (HE, mucin PAS-staining) and immunohistochemical (PCNA and HSP70) analysis, inflammatory (MPO and NAG activity, cytokine levels measurements) and antioxidant (SOD and CAT) parameters were investigated in gastric ulcer tissues.

Results: Oral treatment with SDI accelerated gastric ulcer healing, maintained mucin content and promoted epithelial cell proliferation. SDI also reduced neutrophil and mononuclear leukocyte infiltration, TNF-α and IL-1β levels and the oxidative stress, restoring SOD and CAT activities in the ulcer tissue.

Conclusions: The gastric healing effect of SDI was mediated through endogenous protective events as well as due to the anti-inflammatory and antioxidant actions. Our observations support and reinforce the traditional utilize of *Sedum dendroideum* as a natural nontoxic therapeutic alternative for the treatment of gastric ulcers.

1. Introduction

Peptic ulcer disease affects 10% of the world population (Zapata--Colindres et al., 2006), and is characterized by a deep necrotic injury due to the destruction of mucosal and submucosal layers (Lanas and Chan, 2017). Generally, gastric ulcers develop when an imbalance occurs in the equilibrium between protective (e.g. mucus layer) and aggressive factors (e.g. HCl and pepsin) of the gastric mucosa.

The first-line treatment of peptic ulcers is focused on the suppression of gastric acid secretion through H_2-receptor antagonists (ranitidine) and mainly proton pump inhibitors (omeprazole) (Cryer and Mahaffey, 2014). However, recent research findings reported that the long-term use of gastric acid-suppressive medications promotes drug-related side effects, such as nutritional deficiencies, which are associated with the malabsorption of nutrients, risk of bone fractures and enteric infections with *Clostridium difficile*. All these long-term consequences have been maybe related to the modification of stomach and intestinal pH (Vaezi

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Abbreviations

AChE	Acetylcholinesterase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BSA	Bovine serum albumin
CAT	Catalase
DPPH	Diphenyl-2-picrylhydrazyl radicals
ED50	Effective dose 50
HRP	Horseradish peroxidase
HSP70	Heat Shock Protein 70
IL-1 β	Interleukin 1 beta
MPO	Myeloperoxidase
NAG	N-acetylglucosaminidase
PAS	Periodic acid–Schiff
PCNA	Proliferating cell nuclear antigen
PGE2	Prostaglandin E2
ROS	Reactive oxygen species
SEM	Standard error of mean
SDI	Sedum dendroideum infusion
SOD	Superoxide dismutase
TNF- α	Tumor necrosis factor-alpha
VEGF	Vascular Endothelial Growth Factor

et al., 2017), and for this reason, new alternatives using natural products could decrease drug-induced side effects compared to synthetic compounds.

Sedum dendroideum Moc. & Sessé ex DC. (Crassulaceae) is a succulent plant widely utilized in Brazilian folk medicine for the treatment of gastric ulcers and inflammatory diseases (De Melo et al., 2005). Considering the ethnopharmacological evidence and the profile of active constituents, *Sedum dendroideum* emerges as an important plant that deserves further investigation. In a previous study, our group demonstrated that the *Sedum dendroideum* infusion (SDI) when prepared by soaking the leaves in hot water, displayed gastroprotective effects against acute gastric lesions induced by ethanol, without changes in acid secretion as well as prokinetic activity (da Luz et al., 2019). Nevertheless, despite the ulcer healing properties induced by *Sedum dendroideum,* which has been previously indicated (Carrasco et al., 2014), the mechanisms responsible for gastric ulcer cicatrization have not yet been adequately elucidated. Thus, a chronic experimental model of gastric ulcers in rats was employed to further clarify the potential mechanisms underlying the ulcer healing effects of repeated oral treatments with SDI, rich in phenolic and flavonoids constituents. In addition, to evaluate possible toxic effects in relation to SDI treatments.

2. Materials and methods

2.1. Botanical material and infusion preparation

Sedum dendroideum was harvested in Campina Grande do Sul (25°19′05.3″ S; 49°02′32.3″ W, at 921 m above mean sea level), in the State of Parana (PR), Southern Brazil. Dr. José Tadeu Weidlich Motta, a plant taxonomist and the curator at the Municipal Botanical Museum (MBM) in Curitiba, PR, Brazil, identified the botanical material and a voucher specimen was deposited in the Herbarium Collection of the MBM (MBM-272917). SDI was obtained as previously described (de Oliveira et al., 2018). In brief, the plant was harvested, identified, and then the dried leaves were submitted for extraction using boiling water (100 g/L) by infusion for 1 h. SDI was lyophilized to obtain a dry extract to determine the infusion concentration in order to perform *in vivo* assays. It is noteworthy, that in our previous investigation, the phytochemical composition of SDI was obtained by HPLC–MS analyses and revealed the presence of different flavonol glycosides, containing myricetin and quercetin, along with kaempferol as aglycones (da Luz et al., 2019). The SDI dose employed in this study (191 mg/kg, p.o.) was obtained through the median effective dose (ED_{50}) based on the inhibition of gastric lesions induced by ethanol in rats (da Luz et al., 2019). It is important to highlight that the selected dose to study gastric ulcer healing was made through an ED_{50} calculated dose. This dose was in accordance with the ethnopharmacological use of SDI, following an allometric scaling approach, and thus, converting the human dose into a rat equivalent dose (Nair and Jacob, 2016; da Luz et al., 2019).

2.2. Animals

Female Wistar rats (weight range, 180–200 g), obtained from the Biotery of Federal University of Parana, were kept in plastic cages containing soft pine bedding (maximum of 5 rats per cage) and maintained at 22 ± 2 °C and 12 h-light/dark cycle, with free access to food and water. All animal protocols were approved by the Committee of Animal Experimentation of Federal University of Parana (CEUA/BIO - UFPR: nº 1010) and conducted in agreement with the "Guide for the Care and Use of Laboratory Animals" published by the US National Institute of Health (The National Research Council, 2011).

2.3. Chronic gastric ulcer induced by acetic acid

Chronic gastric ulcers were induced by acetic acid as described previously, with minor modifications (Okabe et al., 1971). All surgical procedures were performed by using aseptic techniques and conducted under operative and post-operative care. Rats were anesthetized with a combination of xylazine and ketamine (10 and 5 mg/kg, i.p. respectively). After a laparotomy, the stomach was exposed and a cylinder (6 mm of diameter) containing a 500 μL solution of 80% glacial acetic acid was applied to the serosal surface of the stomach. After 1 min, the acetic acid was aspirated, the stomach washed with sterile saline and replaced, and the abdomen sutured. Rats were maintained in electric heating blankets until recovery from anesthesia and then returned to their cages. In the present study, only a limited amount of animals experienced intraoperative death (on average, only about 6.66% of animals died during the surgery), and all rats survived until the end of the experiment.

Animals were treated by oral gavage twice daily 1 h after feeding, with water (Vehicle, V: 1 mL/kg), omeprazole (O: 40 mg/kg), sucralfate (S:100 mg/kg), or with SDI (191 mg/kg) for 5 days. On the day following the last treatment, rats were euthanized by thiopental overdose (100 mg/kg, i.p.) and the stomachs were removed and opened to calculate the ulcer area (mm^2), measured as length (mm) × width (mm).

2.4. Histological evaluation

Stomach histology was performed to evaluate microscopic damages induced by an 80% acetic acid solution. The gastric ulcer tissue was fixed with 4% formaldehyde, dehydrated with alcohol and xylene, and embedded in paraffin wax. After that, ulcer tissues were cut with a microtome into 5 μm sections, and stained with hematoxylin/eosin (HE) for a histological evaluation. The ulcer sections were observed and photographed using an Axio Imager Z2 microscope (Carl Zeiss, Jena, DE), equipped with an automated scanning VSlide (MetaSystems, Altlussheim, DE) at × 20 and × 400 magnification. Quantitative histologic assessments including microvessel density and intercapillary distance were measured according to West and coworkers (2001).

2.5. Determination of mucin content

Mucin histochemistry was performed to evaluate the alterations of the mucus content, as previously described (Pereira et al., 2013). Paraffin-embedded sections were deparaffinized, rehydrated, oxidized in 0.5% periodic acid for 5 min, and washed in distilled water. The sections were then stained with Schiff's reagent and subsequently washed with sulphurous water and tap water for 10 min. Periodic acid-Schiff (PAS) positive mucin staining pixel patterns were quantified.

2.6. Immunohistochemical staining

Glass slides were deparaffinized in xylene, rehydrated through a graded series of ethanol, and rinsed with PBS. The antigen retrieval was performed by using a citrate buffer, which blocked endogenous peroxidase activity and prevented the non-specific binding of the antibody to the tissues. Proliferating cell nuclear antigen (PCNA) and 70-kDa heat shock protein 70 (HSP70) were detected during an overnight incubation period at 4 °C in a humidified chamber with the primary antibody goat anti-PCNA (1:100; Santa Cruz Biotechnology Inc., CA, USA) and with primary antibody goat anti-HSP70 (1:100; Santa Cruz Biotechnology Inc., CA, USA). The slides were later rinsed and incubated with a HRP secondary antibody (1:100) at room temperature for 1 h. After washing, the peroxidase-binding sites were detected by staining with DAB (BD Biosciences, California, USA) and counterstained with hematoxylin. Next, the sections were dehydrated and mounted under a cover-slip in Entellan® (Merck, Darmstadt. Germany). PCNA was quantified by counting all positive cells, and HSP70 was quantified by measuring the total staining intensity.

2.7. Preparation of subcellular fractions of stomachs

Stomach samples were homogenized with a cold 200 mM potassium phosphate buffer (pH 6.5) and centrifuged at 9000×*g* for 20 min at 4 °C. Thus, the supernatant was used for the determination of superoxide dismutase (SOD) and catalase (CAT) activity. The pellet was used to determine the myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) activity. To evaluate the tumor necrosis factor-alpha (TNF-α) and interleukin (IL-1β) levels, gastric ulcer samples were homogenized with ice-cold RIPA-buffer (1 mM Tris-HCl pH 7.5, 5 M NaCl, 0.5 M EDTA) containing protease and phosphatase inhibitor cocktails (10 μL/mL) and centrifuged at 9000×*g* for 20 min, and the supernatant was used for analysis. The protein concentration was determined using the Bradford method (Bio-Rad, Hercules, CA, USA), and applying bovine serum albumin as the standard (0.062–1.0 mg/mL).

2.8. Measurement of myeloperoxidase (MPO) activity

Infiltration of neutrophils in gastric ulcers was measured through the MPO activity (Bradley et al., 1982). As mentioned above, the pellet was resuspended in 1 mL of 80 mM potassium phosphate buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide, and once again centrifuged at 11.000×*g* for 20 min at 4 °C. The supernatant was mixed with buffer (0.08 M phosphate buffer, 0.22 M phosphate buffer and 0.017% H_2O_2), and for the colorimetric reaction, 18.4 mM 3,3′, 5, 5′-tetramethylbenzidine was added into the wells of a 96-well plate. The absorbance of the samples was determined at 620 nm, and the results were expressed as units of optic density (O.D.)/mg of protein.

2.9. Measurement of N-acetyl-β-D-glucosaminidase (NAG) activity

The hydrolysis of the substrate p-nitrophenyl-acetyl-β-D-glucosamine by the NAG was used to correlate the presence of mononuclear leukocytes in the gastric ulcers (Bailey, 1988). Samples of the supernatant were incubated with citrate buffer (5 mM, pH 4.5) in the presence of substrate (2.24 mM 4-Nitrophenyl N-acetyl-β-D- glucosaminide). The 96-well plate was incubated at 37 °C for 60 min, followed by the interruption with a 200 mM glycine buffer (200 mM, pH 10.4). The absorbance of the samples was determined at 405 nm, and the results were expressed as units of optic density (O.D.)/mg of protein.

2.10. Measurements of cytokines

Supernatants were used to estimate the TNF-α and IL-1β levels, by using commercial enzyme-linked immunosorbent assay (ELISA) kits, in accordance to the manufacturer's instructions (Peprotech EC Ltd, London UK). The absorbance for TNF-α and IL-1 β was measured at 405 nm with a wavelength correction set at 650 nm and at 450 nm with a wavelength correction set at 620 nm, respectively. Recombinant rat TNF-α standard curves (31.2–3000 pg/mL) and recombinant rat IL-1β standard curves (7.8–4000 pg/mL) were used to interpolate concentrations of all the samples. Results were expressed in pg/mg of protein.

2.11. Prostaglandin E_2 (PGE_2) assay

The PGE_2 levels in plasma were evaluated using a commercial ELISA kit following the manufacturer's protocol (Cayman Chemical, Ann Arbor, MI, USA). With regards to the anesthetized animals, blood was collected through cardiac puncture in vacutainers containing EDTA and 10 μM of indomethacin to prevent *ex vivo* formation of eicosanoids, and plasma was obtained after the centrifugation of blood at 4000×*g* for 5 min. The samples of plasma and standards were added on a 96-well plate with a PGE_2 AChE tracer and PGE_2 monoclonal antibody, and incubated at 4 °C for 18 h. After the plate was washed with wash buffer, and Ellman's Reagent was added and incubated for 60 min, then the absorbance was measured at a wavelength between 405 and 420 nm, the results were expressed in pg/mL.

2.12. Determination of catalase (CAT) activity

The catalase activity was measured by spectrophotometry based on the kinetic decomposition of hydrogen peroxide to water and oxygen (Aebi, 1984). The samples of supernatant were added on a 96-well plate and the enzymatic reaction was initiated by adding the substrate (1 mM Tris EDTA; 5 mM EDTA and 30% H_2O_2). The decomposition of hydrogen peroxide was measured by a spectrometry assay at 240 nm for 1 min and the results were expressed as mmol/mL/min^{-1}.

2.13. Determination of superoxide dismutase (SOD) activity

The method used to determine SOD activity is based on the capacity of SOD to inhibit pyrogallol autoxidation (Moorhouse et al., 1985). Pyrogallol (1 mM) was mixed with a buffer solution (200 mM Tris HCl–EDTA, pH 8.5) and supernatant aliquots, and then vortexed for 1 min. The reaction was incubated for 20 min at room temperature, and the reaction was interrupted by the addition of 1 N HCl. The absorbance was measured at 405 nm. The amount of SOD that inhibited the oxidation of pyrogallol by 50%, relative to the control, was defined as one unit of SOD activity. The enzymatic activity was expressed as U/mg of protein.

2.14. Toxicological profile

During the 5 days of treatments, the body weight was recorded daily. At the end of the treatments, animals were euthanized as previously described. The adrenal glands, heart, kidneys, spleen, ovaries and uterus were removed, weighed, and expressed as the relative organ weights (in relation to body weight). The relative organ weight of each animal was calculated as follows:

Relative organ weight = (organ weight (g)/body weight of the animal on euthanasia day (g)) × 100.

Additionally, the plasma obtained after centrifugation of blood at

4000×*g* for 5 min, was assayed for alanine aminotransferase (ALT), aspartate aminotransferase (AST) (markers of liver damage U/L), and creatinine and urea (markers of kidney injury mg/dL). The parameters were analyzed using an automated system (Mindray BS-200) in accordance with the instructions from the kit manufacturer (Labtest Diagnostica, Brazil).

2.15. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM) and statistical differences between experimental groups were determined using One-way analysis of variance (ANOVA) followed by Bonferroni's multi-comparison post-hoc test. All analyzes were performed with GraphPad Prism® version 6.0 (GraphPad Software, San Diego, USA). Differences were significant when $P \leq 0.05$.

3. Results and discussion

Our results indicate that the oral treatment with SDI accelerated ulcer healing, increased protective factors and decreased several inflammatory and oxidative stress parameters.

In rats, the gastric ulcer model induced by acetic acid is very similar to those observed in humans, resembling characteristics of healing and recurrence. Evaluation of the histological sections demonstrated a penetrating ulcer associated with the destruction of gastric mucosa and muscle tissue, in addition to, leukocyte infiltration and edema formation (Okabe and Amagase, 2005).

The pharmacological therapy for chronic treatment of gastric ulcers involves drugs that enhance mucosal protection or decreased aggressive factors (Tang and Chan, 2012). For this reason, in this study, positive control drugs with different mechanisms of action were selected, such as omeprazole, which inhibits gastric acid secretion and sucralfate, which displays gastroprotective effects by creating a mechanical barrier. Concerning the positive controls, omeprazole and sucralfate significantly reduced the ulcer area by 42.74 and 42.57%, respectively (O: 111.08 ± 3.24 mm2; S: 111.41 ± 8.97 mm2), when compared to the vehicle group (V: 194.0 ± 12.50 mm2) (Fig. 1A–C). Moreover, after 5 days of treatment, SDI accelerated the healing of chronic gastric ulcers in 36.64% (122.9 ± 6.71 mm2) when compared to the vehicle group (Fig. 1A and B). Thus, confirmed through histological slices of gastric ulcers (Fig. 1C). Notably, there were no statistically significant differences among the treated groups.

Histopathological analysis confirms that a repeated treatment with SDI promotes ulcer healing, revealed by the decrease in the diameter of ulcers by 71.50% when compared to the vehicle group (V: 6334.24 ± 899.02 μm). Similarly, the positive controls also decreased the diameter of the ulcers in 78.47 and 65.75% (O: 1363.63 ± 365.92 μm; S: 2169.31 ± 304.36 μm), respectively (Table 1). In addition, SDI also ameliorated parameters related to angiogenesis in the gastric mucosa. The treatment with SDI increased the microvessel density by 57.83% and decreased the intercapillary distance by 49.85% when compared to the vehicle group (V = microvessel density: 7.84 ± 0.60 vessels/field; intercapillary distance: 185.03 ± 7.04 μm), suggesting tissue angiogenesis. Likewise, omeprazole and sucralfate improved the capillary distribution, in both histological parameters indicative of angiogenesis (Table 1). Angiogenesis plays a key role in the process of ulcer healing because regeneration of blood microvessels represents a critical requirement for the removal of aggressive agents and gastric cell renewal (Tarnawski et al., 2014; Ahluwalia et al., 2018).

Table 1
Effect of treatment with SDI on histopathological parameters.

Treatment	Diameter of ulcer (μm)	Intercapillary distance (μm)	Microvessel density (number of vessel/field)
Vehicle	6334.24 ± 899.02	185.03 ± 7.04	7.84 ± 0.60
Omeprazole	1363.63 ± 365.92*	90.56 ± 6.29*	16.42 ± 1.33*
Sucralfate	2169.63 ± 365.92*	103.02 ± 6.75*	16.85 ± 0.89*
SDI	1805.05 ± 302.82*	92.77 ± 4.55*	18.60 ± 0.87*

The results are expressed as mean ± S.E.M. (n = 20). ANOVA followed by Bonferroni's test. *P < 0.05 when compared to the ulcerated vehicle group (V).

Moreover, the histological evaluation also showed a decrease in the amount of mucin-like glycoproteins in the vehicle group. However, with regards to the treatments with omeprazole, sucralfate, and SDI there was an increase in the mucin staining when compared to the vehicle group by 44.02, 53.74, and 52.96% respectively (Fig. 2A and B) (V: 6.35 ± 5.40 pixels/field × 104). The first line of defense against acid is the mucus layer, and the presence of mucin-like glycoproteins supports tissue regeneration and prevents further injury during the healing process (Laine et al., 2008).

Complementarily, the immunohistochemical evaluation of PCNA showed that treatment with SDI increased the number of epithelial proliferating cells, as observed in the control-treated groups (Fig. 2C and D). Interestingly, it is well known that the ulcer healing process also depends on the restoration of mucosal tissue integrity, a fundamental process that implicates in reepithelialization, differentiation, migration, and proliferation of stomach cells (Tarnawski et al., 2014; Ahluwalia et al., 2018). Thus, the treatments with omeprazole, sucralfate, and SDI showed significant increases of 57.89, 61.15, and 52.95% respectively, in the number of PCNA-positive cells when compared to the vehicle group (V: 109.66 ± 3.44 labeled cells) (Fig. 2C and D). Moreover, the immunohistochemical examination of the gastric sections revealed an increase of HSP70 staining by 87.40, 80.99, and 80.53% in the treated groups with omeprazole, sucralfate and SDI respectively, when compared to the vehicle group (V: 11.18 ± 1.66 pixels/field × 104)

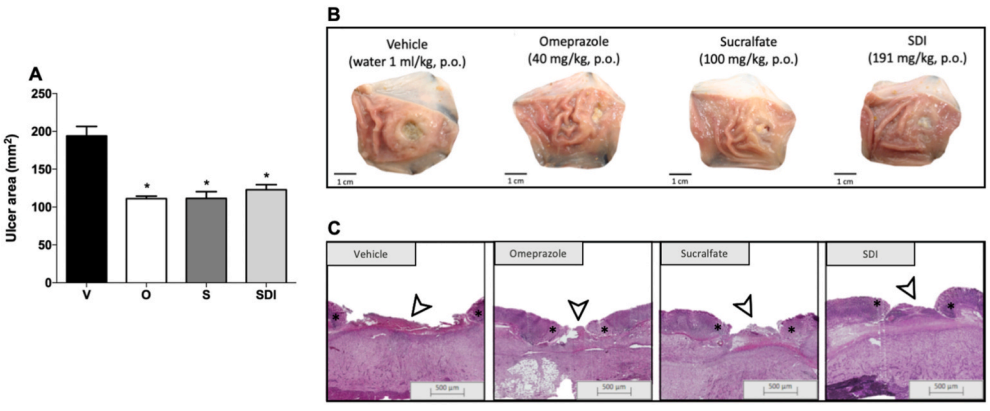

Fig. 1. Effect of oral treatment with SDI 191 mg/kg in chronic gastric ulcer induced by 80% acetic acid in rats (A). The images representing macroscopic photograph (B, Bars = 10 mm) and histological hematoxylin/eosin (HE) sections (C, 20 × , Bars = 500 μm, where * indicates margin of ulcer and ➢ indicates base of ulcer) of ulcerated stomachs. The results are expressed as mean ± S.E.M. (n = 12). ANOVA followed by Bonferroni's test. *P < 0.05 when compared to the ulcerated vehicle group (V).

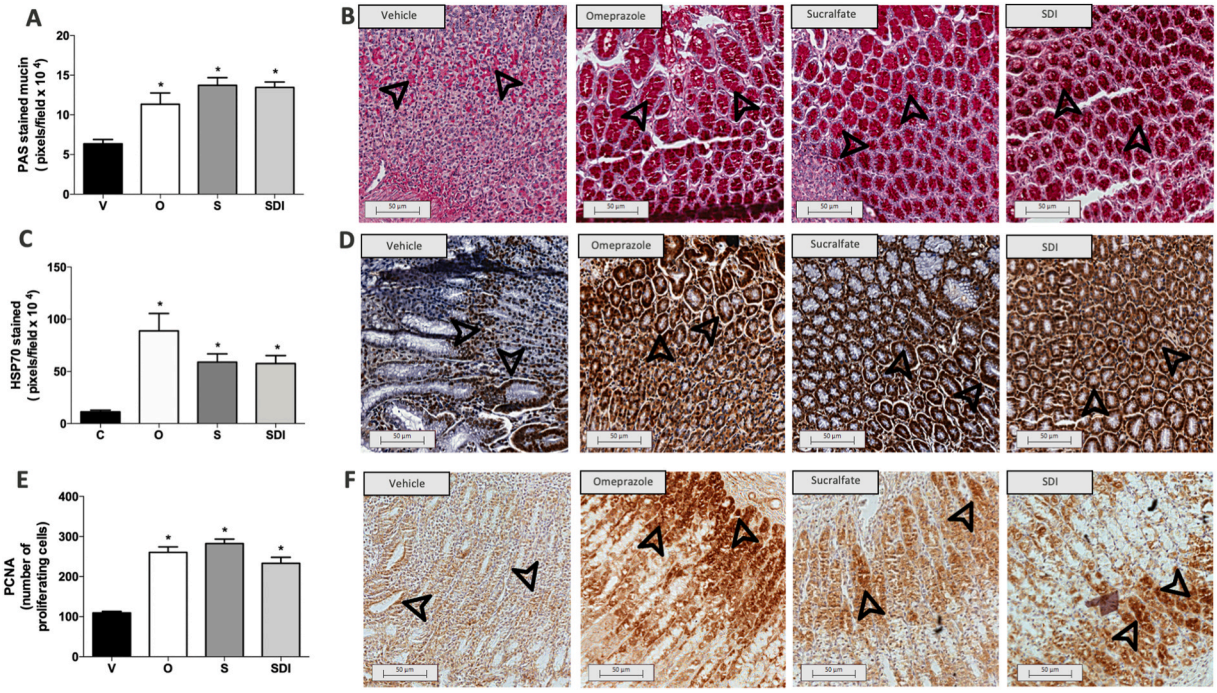

Fig. 2. Effect of oral treatment with SDI 191 mg/kg on the histochemical staining (➢) for mucin-like glycoproteins (PAS) (A and B), immunohistochemical staining for PCNA (C and D) and for HSP70 (E and F) in chronic gastric ulcer induced by 80% acetic acid in rats. Magnification = 400X, bars = 50 μm. The results are expressed as mean ± S.E.M. (n = 12). ANOVA followed by Bonferroni's test. *P < 0.05 when compared to the ulcerated vehicle group (V).

(Fig. 2E and F). The HSP70 are abundant proteins responsible for protecting tissue from thermal or oxidative stress. In the gastric mucosa, they act as a defensive factor through the prevention of ulcer formation, and by supporting the ulcer healing process (Tsukimi et al., 2001; Saremi et al., 2020; El-Shiekh et al., 2021). Our results displayed an increase of HSP70 staining in the treated groups in comparison to the vehicle group (Fig. 2E and F). Thus, indicating that in addition to maintenance of mucin levels and the increase of cell proliferation, the gastric healing promoted by SDI might be attributed to a mucosal defense mechanisms, probably due to cytoprotection provided by HSP70.

Furthermore, the inflammatory response, a prominent finding in the gastric ulcer scar, involves the recruitment of neutrophils and mononuclear cells, which in turn leads to the increase of intracellular reactive oxygen species as well as proinflammatory cytokine production, such as TNF-α and IL-1β (Osawa, 2018). As expected, in the present study, the acetic acid induced-gastric ulcer injury promotes an increase in MPO and NAG enzyme activities in the vehicle-treated group, due to the exacerbated inflammatory response in the stomach (Contreras-Zentella et al., 2017). When compared to the naïve group, MPO and NAG activity increased by 48.81 and 68.30% in the vehicle ulcerated group (N = MPO: 1.18 ± 0.02 O.D./mg of protein; NAG: 36.29 ± 1.23 O.D./mg of protein) (Fig. 3A and B, respectively). The data presented in Fig. 3A shows that animals treated with omeprazole, sucralfate and SDI significantly decreased MPO activity by 58.75, 74.22, and 93.02%, respectively when compared to the vehicle group (V: 3.79 ± 0.69 O.D./mg of protein). Likewise, omeprazole, sucralfate and SDI reduced NAG activity by 32.84, 35.43 and 29.77% when compared to the vehicle group (V: 61.09 ± 5.80 O.D./mg of protein) (Fig. 3B).

In line with these observations, omeprazole, sucralfate and SDI treatments significantly decrease the gastric TNF-α levels by 52.43, 45.66 and 62.43%, when compared to vehicle group (V: 397.69 ± 70.93 pg/mg of protein) (Fig. 3C). In addition, gastric IL-1β levels were reduced by the treatments with omeprazole, sucralfate and SDI by 56.87, 64.95 and 57.75% when compared to the vehicle group (V: 472.39 ± 50.83 pg/mg of tissue) (Fig. 3D). Notably, De Melo and coworkers (2005) found several kaempferol glycosides, being kaempferitrin the most abundant in the fresh juice of *Sedum dendroideum*. This suggests that this chemical profile may explain the popular use against pain and inflammatory diseases. These results are consistent with the previous findings of our research group, where we demonstrated different flavonol glycosides, containing myricetin and quercetin, in addition with kaempferol as aglycones in SDI (da Luz et al., 2019). The remarkable effects of SDI on the control of inflammatory cell infiltration and consequent impairment of the cytokine overexpression may contribute significantly to the healing process of gastric ulcers (Furuta et al., 2002; Watanabe et al., 2002).

As mentioned before, it is well known that angiogenesis significantly influences gastric ulcer healing (Tarnawski et al., 2014; Ahluwalia et al., 2018). Unfortunately, at this moment we are not able to directly access the expression of VEGF in the gastric tissue. However, the increase of endogenous PGE_2 levels was associated with the activation of EP_4 coupled Gs protein receptors. In turn, an increase in the intracellular levels of cAMP, is responsible for the up-regulation of VEGF expression, which is fundamental for the regeneration of gastric lesions (Takeuchi and Amagase, 2018). At the end of the oral treatment with SDI, there was a significant increase of serum PGE_2 levels (39.4%) when compared to the non-treated group (V: 140.06 ± 7.95 pg/mL) (Fig. 3E). Even though our main underlying mechanisms were evaluated, our data remains to be clarified. We suggest that the underlying gastric healing mechanisms following SDI treatment occur due to the stimulation of angiogenesis. This hypothesis is reinforced through the up-regulation of HSP70 that promotes the increase of PGE2 levels, which is able to promote the up-regulation of VEGF expression, and consequently, the tissue repair process (Takeuchi and Amagase, 2018; AlKreathy et al., 2020; Saremi et al., 2020; El-Shiekh et al., 2021). In agreement with this data, it is important to point out that SDI increased the microvessel density, as well as the HSP70 protein expression and the PGE_2 levels, but these mechanisms need to be further investigated.

As previously mentioned, the phytochemical investigation of SDI found a high content of phenolic compounds and flavonoids. In fact, our group previously demonstrated that SDI is present in 2.30% of total phenolic and 1.12% of flavonoid contents. Moreover, we also show an *in*

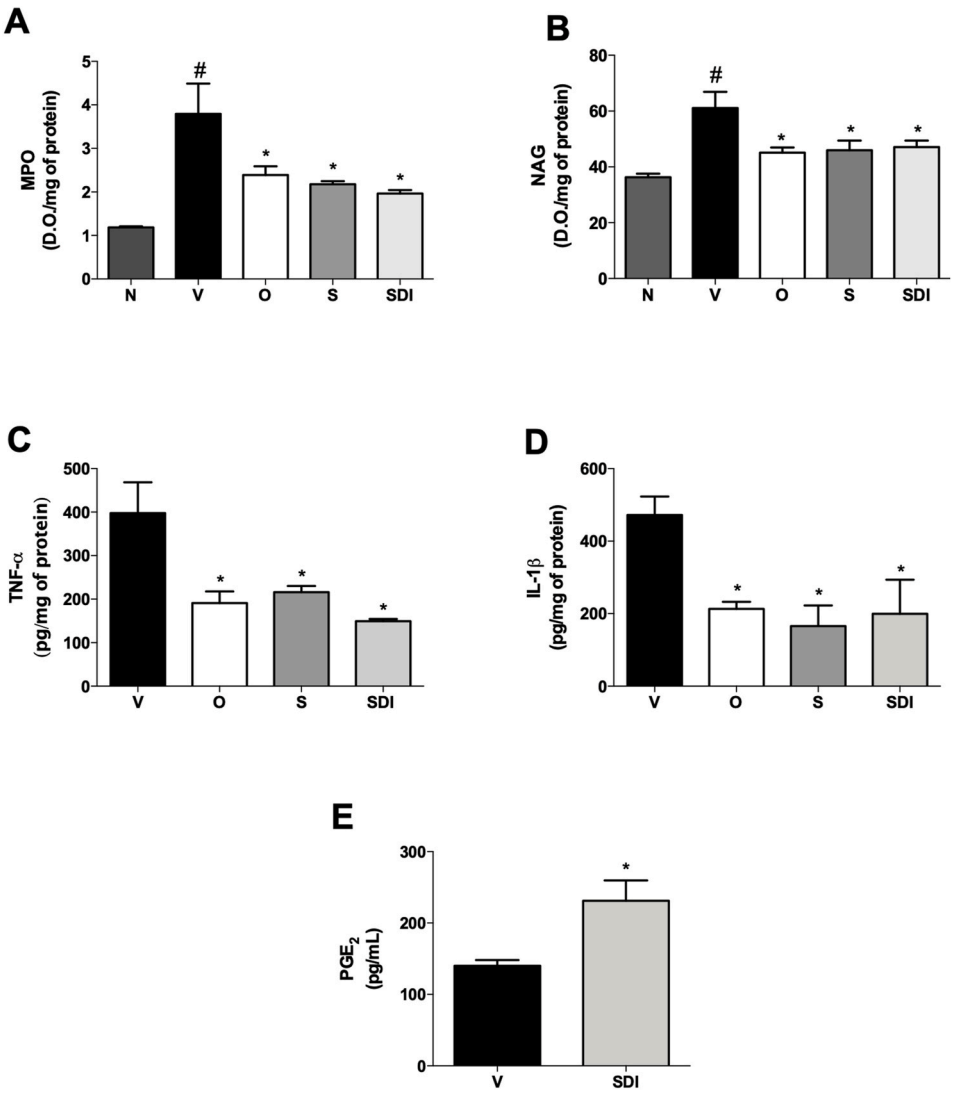

Fig. 3. Effect of oral treatment with SDI 191 mg/kg on inflammatory parameters in chronic gastric ulcer induced by 80% acetic acid in rats. MPO (A) and NAG (B) activity; TNF-α (C), IL-1β (D) and PGE2 (E) levels. The results are expressed as mean ± S.E.M. (n = 6–3). ANOVA followed by Bonferroni's test. *P < 0.05 and #P < 0.05 when compared to the corresponding value of the ulcerated vehicle group (V) or naïve group (N).

vitro free radical scavenging property of SDI in the DPPH assay, suggesting that this plant infusion represents a potent body defense against free radicals (da Luz et al., 2019). There is a general agreement that flavonoids, a group of polyphenolic compounds, possess both free-radical scavenging and anti-inflammatory properties (De Melo et al., 2009). Additionally, Li and collegues (2018) showed that kaempferol, a flavonoid which is present in SDI, promotes gastroprotection through antioxidant and anti-inflammatory mechanisms in a model of ethanol-induced gastric ulcers in mice.

In physiological conditions, it is known that gastric mucosal is normally a key source of reactive oxygen species (ROS) (Aviello and Knaus, 2018). However, unbalanced oxidative stress is considered a pivotal factor in the pathogenesis and maintenance of gastric ulcers, since the overproduction of ROS and free radicals favor the impairment of

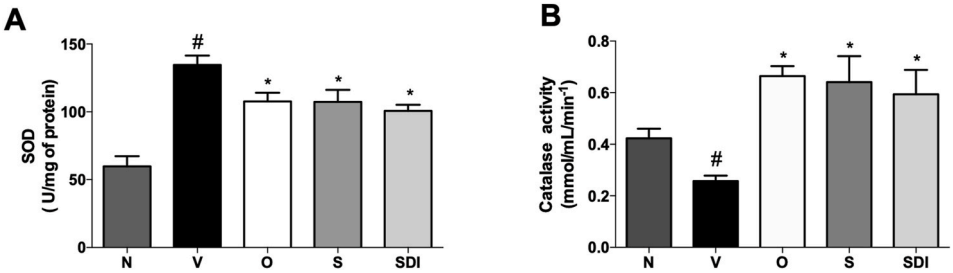

Fig. 4. Effect of oral treatment with SDI 191 mg/kg on antioxidants parameters, represented by SOD (A) and CAT (B) activity in chronic gastric ulcer induced by 80% acetic acid in rats. The results are expressed as mean ± S.E.M. (n = 5). ANOVA followed by Bonferroni's test. *P < 0.05 and #P < 0.05 when compared to the corresponding value of the ulcerated vehicle group (V) or naïve group (N).

stomach antioxidant defense mechanisms (Bhattacharyya et al., 2014). The gastric ulcer induced by acetic acid increased SOD activity by 55.51% when compared to the naïve group (N: 59.86 ± 7.50 U of SOD/mg of protein) (Fig. 4A). The treatment of animals with omeprazole, sucralfate and SDI were able to reverse the increase in SOD activity by 19.99, 20.20 and 25.13%, respectively when compared to the vehicle group (V: 135.55 ± 6.85 U of SOD/mg of protein) (Fig. 4A). The CAT activity was decreased in the ulcerated stomach by 39.28% when compared to the naïve group (N: 0.42 ± 0.03 mmol/mL/min^{-1}). This data could be explained due to the excessive enzymatic activity to remove free radicals, leading to the catalase depletion during the inflammatory process observed in the chronic ulcer model (Murugan and Pari, 2007). Again, animals treated with omeprazole, sucralfate and SDI were able to replace the CAT activity by 61.35, 56.78 and 59.96%, when compared to the vehicle group (V: 0.25 ± 0.02 mmol/mL/min^{-1}) (Fig. 4B). At this point, our results indicate a positive correlation between the reduction of oxidative stress and the ulcer healing promoted by SDI.

Notably, as the acetic acid-induced ulcer model resembles a human chronic ulcer, all parameters evaluated with the antiulcer ED_{50} dose of SDI were quite similar to those obtained with both positive controls. Moreover, as previously demonstrated, SDI does not exert gastric antisecretory effects when compared to omeprazole (da Luz et al., 2019). From the previous findings, SDI did not change the volume, pH and total acidity of gastric hypersecretion in the pylorus-ligated rat model, and as expected, omeprazole decreased the secreted volume, pH and total acidity by 38.97%, 75.10% and 50.25% respectively (da Luz et al., 2019). Interestingly, the SDI single dose (191 mg/kg) employed in the present study was as effective as omeprazole and sucralfate in accelerating the gastric ulcer healing. Notably, SDI at 191 mg/kg present gastroprotective action in ethanol- and indomethacin-induced ulcers without antisecretory effects (da Luz et al., 2019). Taken together, these results demonstrate the effectiveness of *Sedum dendroideum* as a gastroprotective and healing medicinal plant.

Sedum dendroideum is orally consumed in the form of infusion as a popular medicine to treat gastric disorders. Interestingly, non-toxic effects have been reported in the literature in *in vivo* or *in vitro* assays (Carrasco et al., 2014; da Luz et al., 2019). In line with this view, following the repeated treatment with the single dose of SDI (191 mg/kg), no signals of adverse reactions or toxicity, as well as no differences in body weight (Fig. 5) or the ratio organ weight/body weight (Table 2) were observed. Moreover, no changes were observed in the serum toxicological parameters. The levels of ALT, AST, urea and creatinine were within the reference range (Table 3), suggesting a probable safety profile for the intake of SDI.

Thus, the data presented herein, corroborates and extends the findings of Carrasco et al. (2014), revealing the underlying mechanisms behind the healing action displayed by *Sedum dendroideum* in chronic gastric disease. Moreover, different than omeprazole, SDI unchanged the gastric acid secretion (da Luz et al., 2019). These observations reinforce the notion that the mechanisms whereby SDI promotes gastroprotective and healing activities are distinct from those promoted by antisecretory drugs.

4. Conclusion

In conclusion, the results of the present study confirm and reinforce the gastroprotective and healing of injured gastric mucosa properties of *Sedum dendroideum*, contributing to the validation of its popular use (Carlini et al., 1970; Gottschald, 2021). Our findings pointed out that SDI, rich in bioactive compounds, displays gastric health benefits, offering a natural therapeutic alternative for the treatment and healing of gastric ulcers. The gastric ulcer healing promoted by the ED_{50} dose of 191 mg/kg of SDI is due to a resolution of the inflammatory process and oxidative stress, maintenance of gastric mucus and an increase of cell proliferation, without signs of toxicity.

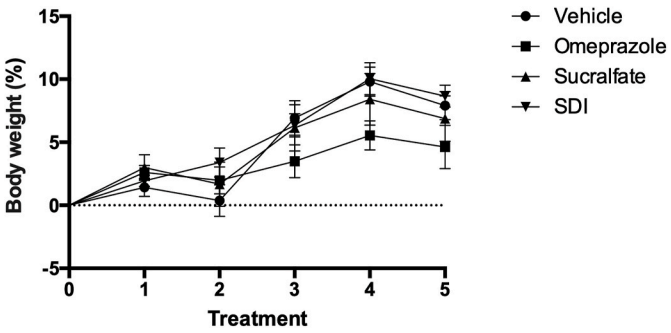

Fig. 5. Effect of oral treatment with SDI 191 mg/kg on the % of body weight. The results are expressed as mean ± S.E.M. (n = 5–7). ANOVA followed by Bonferroni's test. *P < 0.05 when compared to the ulcerated vehicle group (V).

Table 2
Effect of treatment with SDI on the % of body weight relative organ to body weight ratio (g).

Treatment	Adrenals	Spleen	Heart	Ovaries	Kidneys	Uterus
Vehicle	0.021 ± 0.001	0.181 ± 0.013	0.269 ± 0.005	0.021 ± 0.002	0.518 ± 0.006	0.096 ± 0.023
Omeprazole	0.022 ± 0.002	0.194 ± 0.011	0.313 ± 0.013	0.024 ± 0.001	0.513 ± 0.014	0.106 ± 0.019
Sucralfate	0.019 ± 0.002	0.194 ± 0.007	0.290 ± 0.009	0.027 ± 0.001	0.478 ± 0.024	0.126 ± 0.027
SDI	0.020 ± 0.001	0.175 ± 0.015	0.267 ± 0.014	0.020 ± 0.003	0.526 ± 0.018	0.112 ± 0.042

The results are expressed as mean ± S.E.M. (n = 6). ANOVA followed by Bonferroni's test. *P < 0.05 when compared to the ulcerated vehicle group (V).

Table 3
Effect of treatment with SDI on serum biochemical markers.

Treatment	AST (U/L)	ALT (U/L)	Creatinine (mg/dL)	Urea (mg/dL)
Vehicle	160.95 ± 15.34	63.41 ± 2.44	42.20 ± 0.0	13.43 ± 0.37
Omeprazole	178.75 ± 14.85	48.14 ± 4.77	40.24 ± 1.16	14.04 ± 0.81
Sucralfate	110.14 ± 12.93	38.72 ± 2.44	38.29 ± 1.38	14.01 ± 1.30
SDI	164.11 ± 37.93	45.01 ± 5.96	37.02 ± 2.17	12.87 ± 0.55

The results are expressed as mean ± S.E.M. (n = 6). *P < 0.05 when compared to the ulcerated vehicle group (V).

Author contributions

M.F.P.W., B.B.L. and D.M.F. contributed to the concept and design of the study. B.B.L., J.L.D., D.M.F. and M.F.P.W. performed the experiments. T.R.C. and A.F.O. contributed to the obtention of SDI. O.C.B. was responsible for the analysis of renal and hepatic functions. J.E.Q.T. helped with the quantitative histologic measurements. M.F.P.W. and B. B.L. wrote the manuscript. All authors contributed to the analysis and interpretation of data and critically reviewed and approved the final draft.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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