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Antispasmodic, cardioprotective and blood-pressure lowering properties of *Gomphrena perennis* L. and its mechanisms of action

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A B S T R A C T

Background: *Gomphrena perennis* L. is a native plant of South America whose pharmacological properties have not been studied yet.
Aim: To evaluate the cardiovascular and intestinal pharmacological effects of *Gomphrena perennis* L. leaves tincture (GphT) and the mechanisms involved.
Experimental procedure: The chromatographic profile of GphT was done. Its *ex vivo* effects were evaluated by contractile concentration-response curves (CRCs) obtained from the agonist carbachol or calcium found in isolated rat small intestine, as well as in the relaxant CRCs. Cardiac effects were evaluated on isolated rat hearts exposed to ischemia/reperfusion (I/R). Experiments *in vivo* were performed to evaluate the diuretic activity in conscious rats and the hypotensive effect in anaesthetised rats.
Results: Fifteen flavonoids were identified in GphT by HPLC-UV, including diosmin. GphT induced a non-competitive inhibition in both carbachol and calcium CRCs on rat small intestine. The first was not affected by indomethacin. Moreover, GphT, unlike diosmin, relaxed the contracture produced by a high-potassium solution in a dose-dependently way. Neither propranolol nor L-NAME changed it. GphT did not show diuretic activity but induced hypotension insensitive to L-NAME. While GphT perfusion of isolated hearts increased injury consequent to I/R, oral administration was cardioprotective and reversed by L-NAME. However, diosmin did not improve the post-ischemic recovery.
Conclusions: This study supports the use of *Gomphrena perennis* L. tincture as an antispasmodic and hypotensive agent. Moreover, it has been demonstrated to be preventive of post-ischemic cardiac dysfunction. However, diosmin would not be responsible for these effects.

1. Introduction

Despite the therapeutic benefits of current therapies for hypertension

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and gastrointestinal disorders, the incidence of side effects is a frequent problem during treatments. Moreover, patient difficulties in the treatment adherence and costs demand the search of new secure and economical alternatives, and plants are a good option to fulfil those requirements.

Abbreviations

CRC	contractile concentration-response curves
CCh	carbachol
Ca^{2+}	ionic calcium
RRC	relaxation concentration-response
GphT	tincture of *Gomphrena perennis*
EC_{50}	50% effective concentration
IC_{50}	50% inhibitory concentration
HPLC-UV	high-performance liquid chromatography with ultraviolet detection
RP-HPLC	high-performance liquid chromatography in a reverse phase system
% Emax	percentage of the maximal contractile response
NOS	nitric oxide synthase
NO	nitric oxide
iNOS	inducible nitric oxide synthase
NOOX2	NADPH oxidase 2
BP	blood pressure
SBP	systolic blood pressure
DBP	diastolic blood pressure
HR	heart rate
PCIR	postischemic contractile recovery
Ht	total heat rate
P/Ht	total muscle economy
LVP	left ventricular isovolumic pressure
P	maximum developed pressure
LVEDP	or left ventricular end-diastolic pressure

Gomphrena perennis L. (Amaranthaceae) is a native plant from South America popularly known as '*flor de papel*' (literally, 'paper flower') or '*moco-yuyo*'.[1] Anticancer, cytotoxic and antimicrobial activities have been reported for this plant.[2,3] Moreover, several species of the genus *Gomphrena* are used in folk medicine for treating renal, cardiac, central and digestive disorders.[4] There are scientific pharmacological evidences for some other *Gomphrena* species, such as antiarthritic, antihyperalgesic, diuretic and antihypertensive properties of *Gomphrena celosioides,* and the anti-inflammatory effect of *Gomphrena globosa*.[5–7] In addition, the phytocomponents of the other species of *Gomphrena* have been identified and isolated.[8,9]

However, there are no scientific studies that support the ethnopharmacological uses of *Gomphrena perennis* L. in digestive and cardiovascular affections. On the other hand, there are antecedents regarding the traditional antispasmodic uses of the Amaranthaceae family[10] and the cardiovascular properties of the other two species of *Gomphrena*.[6,11] Thus, since it is known that phylogenetically related species produce similar secondary metabolites, we hypothesise that *Gomphrena perennis* L. could also induce antispasmodic, hypotensive and cardioprotective effects. In consequence, the aim of this work was to study whether *Gomphrena perennis* L. tincture could develop such pharmacological activities and to investigate the underlying mechanisms of action. Thus, this is the first scientific study that reports the pharmacological properties of *Gomphrena perennis* L. leaves tincture with *in vivo* and *ex vivo* evidences.

2. Materials and methods

2.1. Preparation of the tincture of *Gomphrena perennis L*

Aerial parts of *Gomphrena perennis* L. were collected in spring from Carlos Spegazzini Botanical Garden and Arboretum, Faculty of Agricultural and Forestry Sciences of the National University of La Plata. The plants were identified by Marta Colares, MSc. Leaves were dried at 40 °C. The hydroalcoholic extract (tincture) of *Gomphrena perennis* L. (GphT) was obtained by maceration of dried leaves at 20% in 70% v/v ethanol (yield = 7.1% w/w extract residue/dried leaves). For *ex vivo* experiments in small intestine, GphT was diluted in Tyrode's and its concentration was expressed as mg of extract residue/mL (mg/mL). For cardiac experiments, GphT was diluted in Krebs-C solution and its concentration was expressed as mg dried leaves/mL. For *in vivo* experiments, GphT doses were expressed as mg of dried leaves/body weight of the animal (mg/kg).

2.2. Chromatographic analysis of *Gomphrena perennis L.* tincture

GphT was analysed by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) using a Dionex Ultimate 3000 UHPLC (Thermo Scientific, Sunnyvale, CA, USA) with a dual gradient ternary pump (DGP-3000) and a diode array detector (DAD-3000), and a manually-operated injector (Rheodyne 7725i, CA, USA) with a 20-μl fixed loop. A C18 column (250 × 4.6 mm, LiChrospher 5 μm, Merck, USA) was used as stationary phase. The mobile phase consisted of a mixture of tetrahydrofuran-methanol-water (15:5:85 v/v) adjusted to pH = 2.5 with phosphoric acid. The system was operated isocratically at room temperature, at a flow rate of 1 ml/min and a detection wavelength of 330 nm. GphT were diluted 1:5 with mobile phase and filtered with a 0.45-μm nylon membrane filter (13 mm, Osmonics Inc., USA) prior to their injection in triplicate. Reference standard solutions of caffeic acid, isoquercetin and rutin were prepared in methanol and then mixed and diluted in mobile phase to yield concentrations of 20, 20 and 10 mg/ml, respectively, in the mixed-standard solution. The sample's chromatogram was analysed by comparing the retention time and DAD spectra (200–400 nm) of the peaks with those of the mixed-standard solution.

Subsequently, chromatographic analyses of flavonoids in GphT were carried out on high-performance liquid chromatography in a reverse phase system (RP-HPLC; binary bomb HPLC, Waters-1525, USA) with a photodiode-array detector (PDA, Waters-2996, USA). A Symmetric 300 (5 μ) reverse-phase C-18 column (250 × 4.6 mm) was used as stationary phase, Waters, USA. The mobile phase consisted of a gradient system composed of a mixture of 0.01 M phosphoric acid (solvent A) and methanol (solvent B). The elution gradient consisted of: a) 70-55% v/v solvent A in solvent B (0–55 min); b) 55-0% solvent A in solvent B (55–95 min); and c) 100% solvent B (95–100 min).[12] The system was operated at 40 °C, at a flow rate of 1 ml/min and the injection volume of 20 μL. Spectral data were recorded in the range 200–700 nm, and chromatograms were recorded at 254, 285 and 365 nm. The chromatograms were analysed by comparing the retention time and the spectral data with those standards.[12,13]

2.3. Pharmacological studies

2.3.1. Animals

Procedures with male and female Sprague-Dawley rats (220–280 g) were developed according to international standards established by US guidelines (NIH publication #85-23 revised in 1996). A local ethical committee of the Faculty of Exact Sciences of the National University of La Plata approved the protocols (015-05-15, D-01-15-16 and 001-00-

18). In the *ex vivo* experiments (isolated small intestine), rats were deprived of food, but they were allowed to drink water *ad libitum* 12 h before the experiments.

2.3.2. Isolated intestinal muscle preparation and contractile measurements

Rats were anaesthetised with a pentobarbital overdose (60 mg/kg, i.p.) and tramadol (10–20 mg/kg, sc). About 2 cm long portions of duodenum and ileum were mounted in organ baths containing constantly oxygenated Tyrode's solution (20 ml, pH 8.2) at 37 °C.[14,15] Isolated tissues were longitudinally connected to an isometric transducer (WPI, USA) and equilibrated at 1g of preload for 45 min. The signals were simultaneously amplified using a 4-channel preamplifier (WPI, USA) and recorded with Eagle software (USA) or PowerLab 2/26 data acquisition system (AD Instruments, Australia).

2.3.3. Carbachol concentration-response curves (CCh-CRCs)

CCh-CRCs were performed on isolated rat small intestine portions by cumulatively adding the dilutions to the bath (range 0.01–10 μg/mL) in the absence (control condition) and presence of the ethanolic vehicle (control vehicle, 70% v/v ethanol diluted similarly to GphT treatment, up to a maximal concentration of 0.7% v/v ethanol) (Fig. A1). In the presence of vehicle, CRCs did not show differences compared to control conditions, so the contractile effect of each CCh concentration was expressed as the percentage of the maximal contractile response (% Emax) under control condition. Following the vehicle-CRCs, a series of curves were obtained in the presence of a single and growing-order concentration of GphT (from 0.14 to 14.05 mg extract/mL) by adding it 5 min prior to the CCh-CRCs. In order to evaluate whether the mechanism involved prostaglandins release, the same protocols were followed in the presence of indomethacin 0.1 μmol/L, a cyclooxygenase inhibitor.

2.3.4. Calcium concentration-response curves (Ca^{2+}-CRCs)

Ca^{2+}-CRCs were performed on isolated rat duodenum and ileum portions by cumulatively adding $CaCl_2$ (range 0.0195–17.5 mmol/L) in a depolarizing media. After stabilisation, Ca^{2+} was removed by replacing Tyrode's solution with Ca^{2+}-free Tyrode's one. Then, tissues were depolarized with 40 mmol/L K^+ and the control Ca^{2+}-CRCs was done. After washing with Ca^{2+}-free medium, vehicle (ethanol up to a maximal concentration of 2.1% v/v ethanol) and, afterwards, GphT single concentrations in growing order (from 0.14 to 28.1 mg extract/mL) were individually added to the bath 5 min before Ca^{2+}-CRCs. The % Emax was measured with the same approach previously described, as there were no differences in the contractile effect in the presence or absence of vehicle (Fig. A2).

2.3.5. Relaxation response concentration (RRC) curves of Gomphrena perennis L. tincture

After contracting intestinal tissue with 40 mmol/L ClK in Tyrode solution, RRC curves were obtained on the tonic phase by adding consequently several GphT concentrations in growing order (from 0.14 to 28.1 mg extract/mL) (Fig. A3). The same protocol was followed in the presence of 30 μmol/L N^{ω}-nitro-L-arginine methyl ester (L-NAME) to inhibit the nitric oxide synthases (NOS)[16] or with 1 μmol/L propranolol to block β-adrenergic receptors.[17] In addition, another RRC curves were obtained by adding diosmin concentrations in growing order (from 10 to 300 μM).[18] (Fig. A4a).

2.3.6. Parameters of CRCs

The pEC_{50} values of the agonists were calculated as –log EC_{50} in mol/L.[19] The 50% inhibitory concentration of the extract GphT (IC_{50} in mg extract/mL) was obtained by interpolation at the 50% effect of the individual inhibitory curves, as previously described.[15]

2.3.7. Measurement of blood pressure (BP) and heart rate (HR)

Normotensive rats were anaesthetised with urethane (1.5 g/kg, i.p.) and placed in a supine position with tracheal cannulation. The jugular vein was cannulated to administer treatments in volumes of 0.1 ml. BP was directly measured with a heparinised cannula in the internal carotid artery connected to a Bentley 800 pressure transducer.[21] The waves of BP were continuously recorded by PowerLab 2/26 data acquisition system (AD Instruments, Australia), from which the minimum (diastolic blood pressure, DBP) and maximum (systolic blood pressure, SBP) levels were measured, and the heart rate (HR) was calculated. After 30 min of stabilisation, negative control of saline followed by doses of 2.5, 10 and 25 mg dried leaves/kg GphT were assessed, all of them at intervals of at least 30 min for stabilisation (Fig. A5). After that series, L-NAME (25 μg/kg) was intravenously administered. When DBP and SBP were stabilised once again, 25 mg dried leaves/kg GphT were newly assessed. In other group of five rats, the diluted ethanolic vehicle (5% v/v) was evaluated.

2.3.8. Diuretic activity of Gomphrena perennis L. tincture

The diuretic effect of GphT was investigated in other group of rats, as previously described.[20] Briefly, 24 h before the experiment, they were given water *ad libitum* and deprived of food. Four pairs of rats were orally administered with the following treatment (in volumes of 4 mL/rat): saline solution (NaCl 0.9%, as negative control), 10 mg/kg amiloride (as positive control) or 100 and 400 mg/kg GphT. Each pair of rats was kept in their respective metabolic cages without food or water, and their urine volumes were measured every 30 min for 5.5 h. At the end of this period, an aliquot was extracted for quantification of Na^+ and K^+ content (in mEq/kg rat weight) by flame photometry.[20] The protocol was repeated in intervals of one week while the treatments of each rat were randomised up to obtain n = 4 in each treatment group. The urinary volumetric excretion (UVE %) was calculated as the ratio between the collected urine volume (mL/kg rat weight) and the administered solution volume (mL/kg rat weight) × 100.[21]

2.3.9. Isolated heart preparation with mechanical and energetic measurements

Rats were heparinised (non-fractionated 2000 IU) and anaesthetised with a pentobarbital overdose (60 mg/kg; i.p.) and tramadol (10–20 mg/kg; s.c.). Hearts were rapidly excised and perfused through coronary arteries by the Langendorff method with control Krebs solution (Krebs-C) by using a peristaltic pump (Gilson Minipuls, France) at 37 °C and a constant flow rate of 7 mL/min/g. This flow rate was calculated according to the equation $CF = 7.43 \times HW^{0.56}$ (where CF means coronary flow and HW means heart weight), in order to prevent cardiac oedema typical of a high flow of saline perfusion.[22] After removing the atria to interrupt spontaneous beating, a latex balloon with a cannula full of water was inserted through the opened atria-ventricular hole into the left ventricle. It was sutured, in a way that the cannula exited from ventricle to be connected to a Bentley 900 pressure transducer. Then, the perfused ventricles were introduced into the chamber of a flow calorimeter which was closed, and vertically submerged in a water bath at 37.0 ± 0.01 °C.[23] Perfusion freely dripped into the draining without being accumulated in the ventricle around the balloon (Fig. A6a). After that, ventricles were electrically stimulated with 5 V for 5 ms at 3 Hz by means of a Letica LE12406 (Spain) electrical stimulator, and the intraventricular ball was inflated in steps to obtain the better developed pressure of contraction. The signals of total heat rate (Ht) and left ventricular isovolumic pressure (LVP) were simultaneously and continuously recorded in mV by a PowerLab 2/26 digital acquisition system (AD Instruments, Australia). The LVP signal was calibrated in mmHg, and the maximum developed pressure (P) of a contraction was calculated from the difference between the LVP peak and the left ventricular end-diastolic pressure (LVEDP), adjusted at the beginning of the experiment (Fig. A6b). The Ht released by heart was calculated from the difference between the signal with muscle and the baselines (without muscle), respectively with and without perfusion, and finally expressed in mW/g wet weight, after considering the respective calibration

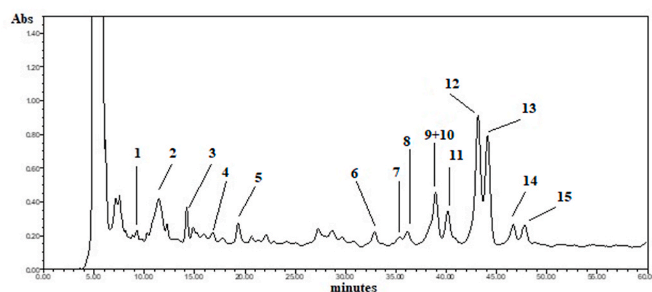


Fig. 1. Chromatographic profile at 285 nm of the GphT determined by RP-HPLC-DAD.
[1. gallic acid; 2. (+)-catechin; 3. eriocitrin (eriodictyol-7-β-rutinoside); 4. neoeriocitrin (eriodictyol-7-β-neohesperidoside); 5. p-coumaric acid; 6. isorhoifolin (apigenin-7-β-rutinoside); 7. acacetin-7-O-[rhamnosyl(1–2)glucoside]; 8. isovitexin (6-C-glucosylapigenin); 9. rhoifolin (apigenin-7-β-neohesperidoside/apigenin-7-O-rhamnoglucoside); 10. 8-hydroxyluteolin-7-O-glucoside; 11. diosmin (diosmetin-7-β-rutinoside); 12. scaposin (5,7-dihydroxy-2-(3-hydroxy-4,5-dimethoxyphenyl)-6,8-dimethoxychromen-4-one); 13. apigenin glycoside; 14. baicalin (baicalein 7-O-β-D-glucuronide); 15. 7-O-methylherbacetin glycoside].

factor.[23] Moreover, P and Ht were expressed as a percentage of the initial value, whereas total muscle economy was calculated as the P/Ht ratio.

2.3.10. Cardiac experimental protocols

Cardiac effects of GphT were assessed by sequentially perfusing it at a concentration of 0.1, 0.3 and 0.5% v/v in Krebs-C for 15 min each one after stabilisation with Krebs-C. P and Ht were continuously recorded. Moreover, the effects of GphT on ischemic heart were evaluated. Isolated rat hearts were exposed to ischemia/reperfusion model (I/R = 20 min of global no-flow ischemia followed by 45-min reperfusion with Krebs-C) as previously described.[23] The following protocols were done (Fig. A7): a) GphT at 0.1% v/v was perfused for 15 min prior to I/R; b) oral 25 mg GphT/kg/day was administered in the drinking water to rats for a week, and afterwards, isolated hearts were exposed to I/R without or with perfusion of 30 μmol/L L-NAME during 15 min before I/R; and c) oral 100 mg diosmin/kg/day was administered in the drinking water to a group of rats for a week prior, and finally, isolated hearts were exposed to I/R.

2.3.11. Measurement of infarcted area

Infarcted area of some of the reperfused hearts was calculated and expressed as a percentage of the left ventricular area, with triphenyltetrazolium chloride staining. The method was previously described.[23]

2.3.12. Drugs and solutions

The composition medium, solution and drugs employed are described in Appendix A.

2.3.13. Statistical analysis

Results were expressed as the mean ± SEM (standard error of the mean; n: number of experiments). Multiple comparisons were analysed by two-way ANOVA or by one-way ANOVA test, as appropriate Tukey's paired *post hoc* tests were done between the multiple treatments. $P < 0.05$ was considered the level of significance. Statistical analyses were performed by using GraphPad Prism 8.0 software.

3. Results

3.1. Chromatographic characterization of the plant extracts

Chromatographic profile of extracts of *Gomphrena perennis* L. tincture (GphT) obtained by high-performance liquid chromatography (HPLC) showed three peaks which did not correspond to the reference substances (Fig. A8a, Table A1). Furthermore, UV spectra of the peaks exhibited two bands of maximum absorption in the ranges of 240 nm–280 nm (band I) and 330 nm–350 nm (band II), respectively (Fig. A8b).

Table 1
RP-HPLC-DAD identification of flavonoids in *Gomphrena perennis* tincture tincture. Retention time (min) and maximum absorption wavelength (λ_{max}, nm) of identificated flavonoids.

Peak	Metabolite	Retentiontime (min)	λ_{max} (nm)
#1	gallic acid	8.8	276
#2	(+)-catechin	10.8	280
#3	Eriocitrin	14.9	282
#4	Neoeriocitrin	16.7	283
#5	*p*-coumaric acid	19.3	310
#6	Isorhoifolin	32.8	275–332
#7	acacetin-7-*O*-[rhamnosyl(1–2) glucoside]	35.3	275–328
#8	Isovitexin	36.1	272–329
#9	Rhoifolin	38.3	274–334
#10	8-hydroxyluteolin-7-*O*-glucoside	38.9	275–340
#11	Diosmin	40.1	275–344
#12	Scaposin	43.1	280–333
#13	apigenin glycoside	44.1	280–336
#14	Baicalin	46.6	279–321
#15	**7-*O*-methylherbacetin glycoside**	**47.8**	**280–319**

The phenolic composition of GphT was determined by RP-HPLC-DAD (Fig. 1). The identified flavonoids showed UV absorption maxima with the two characteristic bands at 272–280 and 319–344 nm. The UV absorption maxima corresponding to the determined phenolic acids were also observed. Figs. A9 and A10 show the UV spectra obtained in the mobile phase used. Table A1 shows the retention time and the maximum absorption wavelength (λ_{max}) of identified flavonoids (Table 1).

3.2. Antispasmodic effects of Gomphrena perennis L. tincture on intestinal smooth muscle

GphT significantly inhibited the CCh-CRCs ($pEC_{50} = 6.2 \pm 0.10$, n = 7) in rat duodenum and ileum as a non-competitive antagonist in a concentration-dependent way (Fig. 2a) with an IC_{50} of 2.68 ± 0.74 mg extract/mL (Fig. A11a). In order to evaluate the mechanism of action, other CCh-CRCs were developed in the presence of 0.1 μmol/L indomethacin (Fig. 2b). Indomethacin did not significantly change the IC_{50} of GphT (2.15 ± 0.39 mg extract/mL, Fig. A11b). Results suggested that the antispasmodic effect of GphT was not due to the release of intestinal prostaglandins.

GphT also inhibited the Ca^{2+}-CRCs ($pEC_{50} = 2.47 \pm 0.06$, n = 6) as a non-competitive antagonist (Fig. 2c) with an IC_{50} of 21.61 ± 7.46 mg extract/mL (Fig. A11c). Vehicle did not significantly change the CRCs vs. control. To investigate other involved mechanisms, relaxation response curves of GphT (GphT-RRC) were determined in the absence and the presence of 1 μmol/L propranolol or 30 μmol/L L-NAME. Neither propranolol nor L-NAME significantly changed the GphT-RRC ($pEC_{50} = 4.64 \pm 0.72$, n = 6) (Fig. 2d). Results suggested that the relaxant effect of GphT was not due to direct activation of β_2-adrenergic receptors or NOS in isolated rat small intestine.

Moreover, the identified as present compound diosmin did not change the intestinal contracture induced by high-[K^+] (Fig. A4b).

3.3. Effects of Gomphrena perennis L. on blood pressure

Two groups of normotensive rats were evaluated. In the first group, saline and three doses of GphT (0.25, 10 and 25 mg/kg) were sequentially administered. The vehicle was administered in the second group of rats. Only 10 and 25 mg dried leaves/kg of GphT significantly reduced

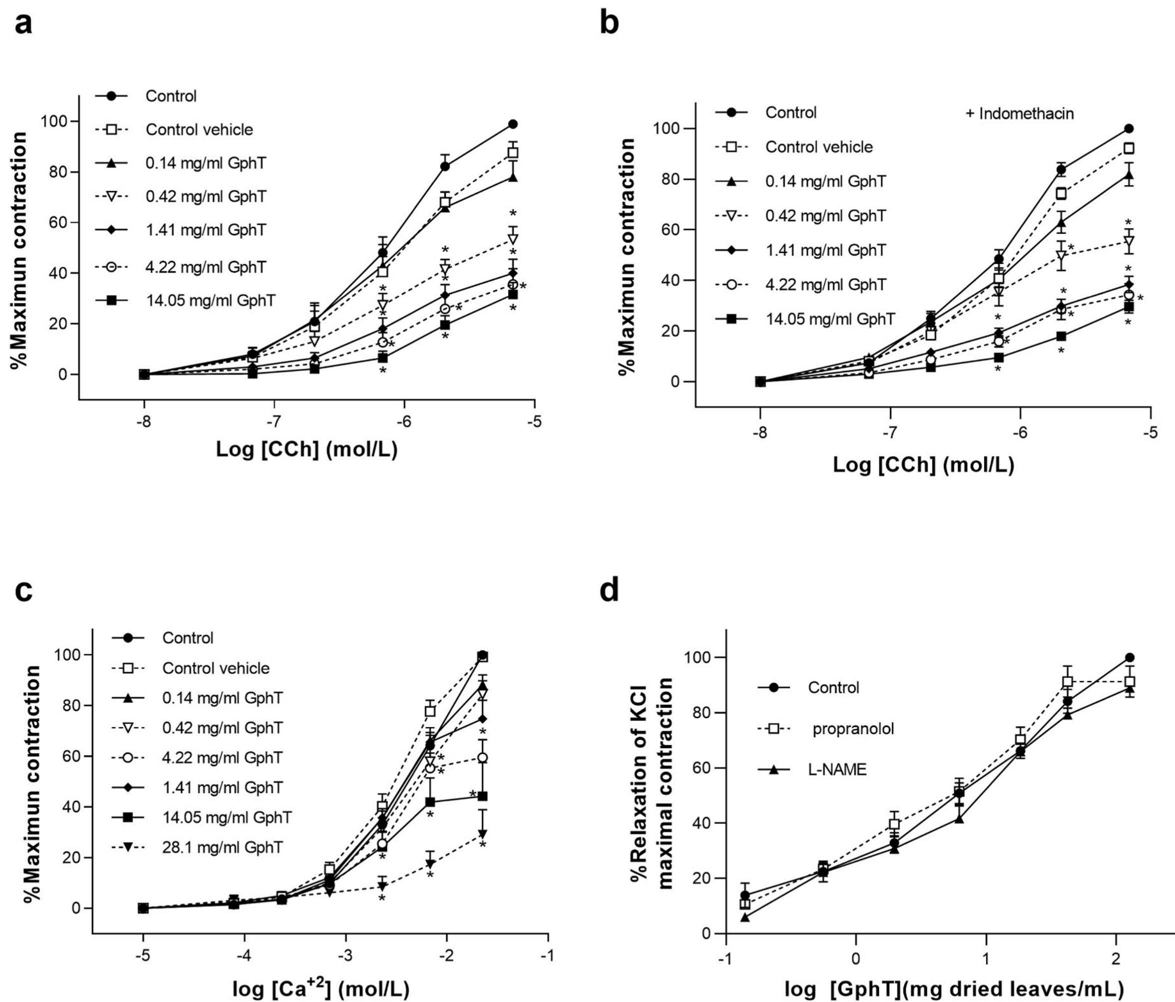


Fig. 2. Effects of *Gomphrena perennis* L. tincture (GphT) on the contractile concentration-response curves (CRCs) of carbachol (CCh) (a) without indomethacin, (b) with indomethacin, (c) on CRCs of $CaCl_2$, and (d) the relaxation response curve of GphT in isolated rat small intestine. Two-way ANOVA (Table A3). *A posteriori* Tukey's test: *p < 0.05 vs. control.

the DBP and the SBP regarding saline at 210.3 ± 25.9 s after infusion without changes on HR. Neither saline nor vehicle significantly changed basal parameters. After the GphT series, the same rats were treated with 25 μmol/kg L-NAME (competitive NOS blocker). L-NAME increased SBP and DBP without changes on HR but it did not prevent the hypotension induced by 25 mg dried leaves/kg of GphT (Table 2). Thus, the hypotensive effect of GphT was not mediated by nitric oxide (NO) release.

3.4. Diuretic activity

Total urine volume was not increased by GphT at doses of 100 and 300 mg dried leaves/kg in comparison to vehicle (Fig. 3a), while only amiloride (10 mg/kg) induced a urinary volumetric excretion significantly higher than 100% (Table A2). Additionally, GphT did not change the urinary ionic excretion (Fig. 3b). Therefore, GphT did not produce diuretic activity at the assessed doses.

3.5. Effects of Gomphrena perennis L. on isolated rat hearts

Ventricles perfused with Krebs-C developed a contractile maximal pressure (P) of 85.33 ± 6.35 mmHg and a total heat rate (Ht) of 17.50 ± 2.18 mW/g, while LVEDP reached 10.72 ± 1.78 mmHg (n = 12). Perfusion of 0.1% v/v GphT induced a slightly positive inotropic effect and significantly increased muscle economy (P/Ht). However, P fell with increasing GphT concentration while the vehicle did not change P or Ht (Table 3). When 0.1% v/v GphT was perfused prior to ischemia, the postischemic contractile recovery (PICR) and total muscle economy (P/Ht) fell drastically with a raised diastolic pressure (ΔLVEDP) during reperfusion (Fig. 4).

However, oral administration of 25 mg/kg/day GphT for seven days increased the PICR and P/Ht without significant changes in the ΔLVEDP during I/R. Moreover, when the NOS were inhibited by perfusing 30 μmol/L L-NAME before I, the beneficial effects of oral treatment were abolished. Results suggested that NO production had a role in GphT-induced cardioprotection. On the other hand, isolated hearts from diosmin oral treatment induced less PICR and P/Ht recovery than oral GphT (Fig. 4).Neither control heart nor oral GphT or diosmin developed significant infarct size areas after I/R (Fig. A12).

4. Discussion

This was the first report demonstrating the hypotensive, cardioprotective and antispasmodic effects of the tincture of *Gomphrena perennis* L. (GphT) in preclinical models from *in vivo* and *ex vivo* preparations and the mechanisms of action involved in them. Moreover, it rejected the possible diuretic activity of this plant under the conditions tested.

The spasmolytic effect of GphT was shown on isolated rat small intestine. The GphT inhibited the CCh-CRCs in a non-competitively way, suggesting that the extract acted at a different site from the muscarinic

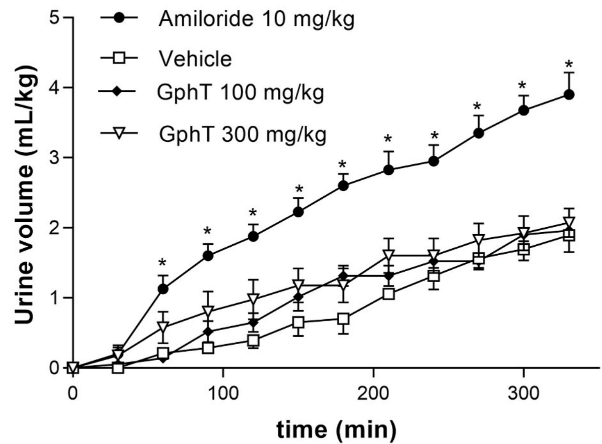
Table 2
Effects of *Gomphrena perennis* L. tincture (GphT) on blood pressure and heart rate of anaesthetised normotensive rats.

Treatment	DBP (mmHg)	SBP (mmHg)	MBP (mmHg)	ΔDBP (mmHg)	ΔSBP (mmHg)	HR (Hz)
Basal (n = 4)	96.41 ± 10.26	106.69 ± 9.52	99.83 ± 9.96	–	–	334.5 ± 20.40
Salina (n = 4)	95.57 ± 10.57	104.35 ± 9.23	98.50 ± 10.01	−0.83 ± 1.12	−2.34 ± 1.80	330.2 ± 10.02
Vehicule (n = 5)	95.84 ± 10.95	111.04 ± 13.43	100.91 ± 11.66	1.21 ± 0.24	1.49 ± 2.01	310.8 ± 8.01
GphT 2.5 mg/kg (n = 4)	81.16 ± 10.9	91.27 ± 9.49	84.53 ± 10.33	−15.24 ± 0.76	−15.42 ± 2.41	336.0 ± 20.28
GphT 10 mg/kg (n = 4)	70.41 ± 10.92	79.58 ± 8.79	73.47 ± 10.21	−25.99 ± 7.07[a]	−27.11 ± 5.18[a]	325.5 ± 23.79
GphT 25 mg/kg (n = 4)	73.04 ± 12.32	82.33 ± 11.22	76.14 ± 11.91	−23.36 ± 8.27[a]	−24.36 ± 6.05[a]	324.0 ± 28.81
GphT 25 mg/kg + l-NAME (n = 4)	73.90 ± 14.67	80.86 ± 12.50	76.22 ± 13.63	−22.50 ± 10.84	−14.69 ± 9.99[a]	336.0 ± 21.17
One-way ANOVA	F = 1.206 P = 0.3403 DFn = 6 DFd = 22	F = 1.523 P = 0.2172 DFn = 6 DFd = 22	F = 1.330 P = 0.2859 DFn = 6 DFd = 22	F = 1.330 P = 0.2859 DFn = 6 DFd = 22	F = 9.927 P < 0.0001 DFn = 5 DFd = 19	F = 0.2402 P = 0.9582 DFn = 6 DFd = 22

[a] $p < 0.05$ vs saline by *a posteriori* Tukey's tests.

receptors. Considering that the indomethacin did not modify the CCh-CRCs, the antispasmodic effect of GphT was not mediated by prostaglandin release. GphT also inhibited the Ca^{2+}-CRCs in a non-competitive way, in a depolarizing condition known to be dependent on Ca^{2+} influx through L-type-Ca^{2+} channels. In this regard, verapamil, a Ca^{2+} channels blocker, also showed a non-competitive effect on cholinergic and high [K^+]-induced contractions in isolated rabbit jejunum and rat small intestine, as GphT did.[15,24] Accordingly, the GphT directly relaxed the intestinal contracture induced by high-[K^+], as other calcium channel blockers did on different smooth muscles.[25,26] Moreover, the addition of propranolol (a non-selective β-adrenergic blocker that reduces the cAMP and PKA pathways)[27,28] or L-NAME (NOS inhibitor that prevents the activation of cGMP-dependent relaxation)[26] did not significantly change the GphT-RRCs. Thus, the antispasmodic effects of GphT seemed to be due to Ca^{2+}-channels blocking but not to activation of β_2-adrenergic receptor or NO release.

The HPLC-UV characterisation allowed us to dismiss the presence of caffeic acid, rutin and isoquercetin in GphT. Also, the GphT showed a complex phenolic profile by highlighting flavonoids, particularly glycosylated flavones. It was possible to identify glycosylated derivatives of apigenin and luteolin, as well as the flavanones eriocitrin and neoeriocitrin. The presence of (+)-catechin, phenolic acids gallic, isovitexin, diosmin and *p*-coumaric was also demonstrated. Several reports about flavonoids showed their spasmolytic effect in intestinal smooth muscle by blocking Ca^{2+} channels.[29,30] Besides, flavones inhibited the responses to acetylcholine, histamine and noradrenaline in the same tissue.[31] Thus, the flavones present in GphT could contribute to its antispasmodic effect. As previously reported[18] we showed that diosmin did not exert intestinal smooth muscle relaxing effects. Similarly, we have previously shown that isovitexin lacked this activity in rat duodenums.[14] Contrary, apigenin showed spasmolytic effect on bladder smooth muscle by several mechanisms including calcium channel blockade.[32] Therefore; since the contractile mechanisms of smooth muscle are similar, the



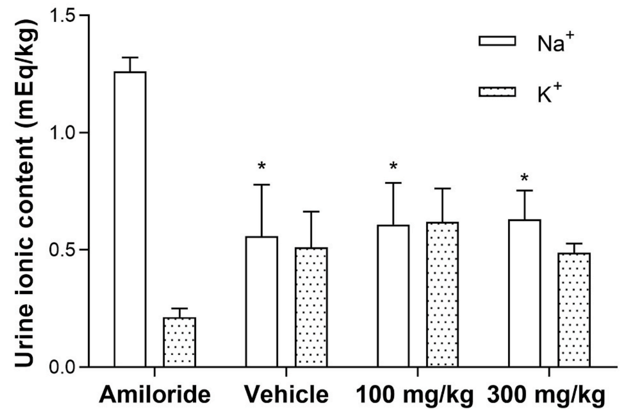


Fig. 3. Effect of *Gomphrena perennis* L. tincture (GphT) on (a) cumulative urinary volume in a single-dose model and (b) urinary ionic contents (mEq/kg) after 5.5 h of diuresis. Two-way ANOVA (Table A4). A posteriori Tukey's test: #p < 0.05 vs treatment and vehicle; #p < 0.05 vs. amiloride.

Table 3
Effect of direct perfusion of growing concentrations of *Gomphrena perennis* L. tincture (GphT) on contractile (P) and energetic (total heat rate and muscle economy) parameters of isolated rat hearts.

Treatment	P (mmHg)	Ht (mW.g^{-1})	P/Ht (mmHg.g.mW^{-1})
Control (n = 6)	73.64 ± 5.69	12.94 ± 2.17	6.28 ± 0.85
Vehicle (n = 6)	75.65 ± 3.64	12.89 ± 2.03	6.46 ± 0.82
GphT 0.1% (n = 6)	87.94 ± 3.79	11.91 ± 1.66	7.89 ± 0.74
GphT 0.3% (n = 6)	70.56 ± 4.23	13.94 ± 1.53	5.29 ± 0.57
GphT 0.5% (n = 6)	51.83 ± 7.25[a]	11.88 ± 1.81	4.41 ± 0.27
One-way ANOVA	**P = 0.0420 F = 2.906 DFn = 4 DFd = 25**	**P = 0.9289 F = 0.2127 DFn = 4 DFd = 25**	**P = 0.2057 F = 1.599 DFn = 4 DFd = 25**

[a] $p < 0.05$ vs GphT 0.1% by *a posteriori* Tukey's tests.

apigenin glycoside found in GphT could be at least partially responsible for its intestinal relaxing effects.

Furthermore, the acute administration of GphT reduced blood pressure without diuretic activity *Gomphrena celosioides* showed antihypertensive and diuretic properties related to the inhibition of the angiotensin-converting enzyme and NO release, as well as antioxidant activity.[5] Regarding the components found in GphT, rhoifolin showed an antihypertensive effect in dogs[33] and *in vitro* ACE inhibitory activity[34]

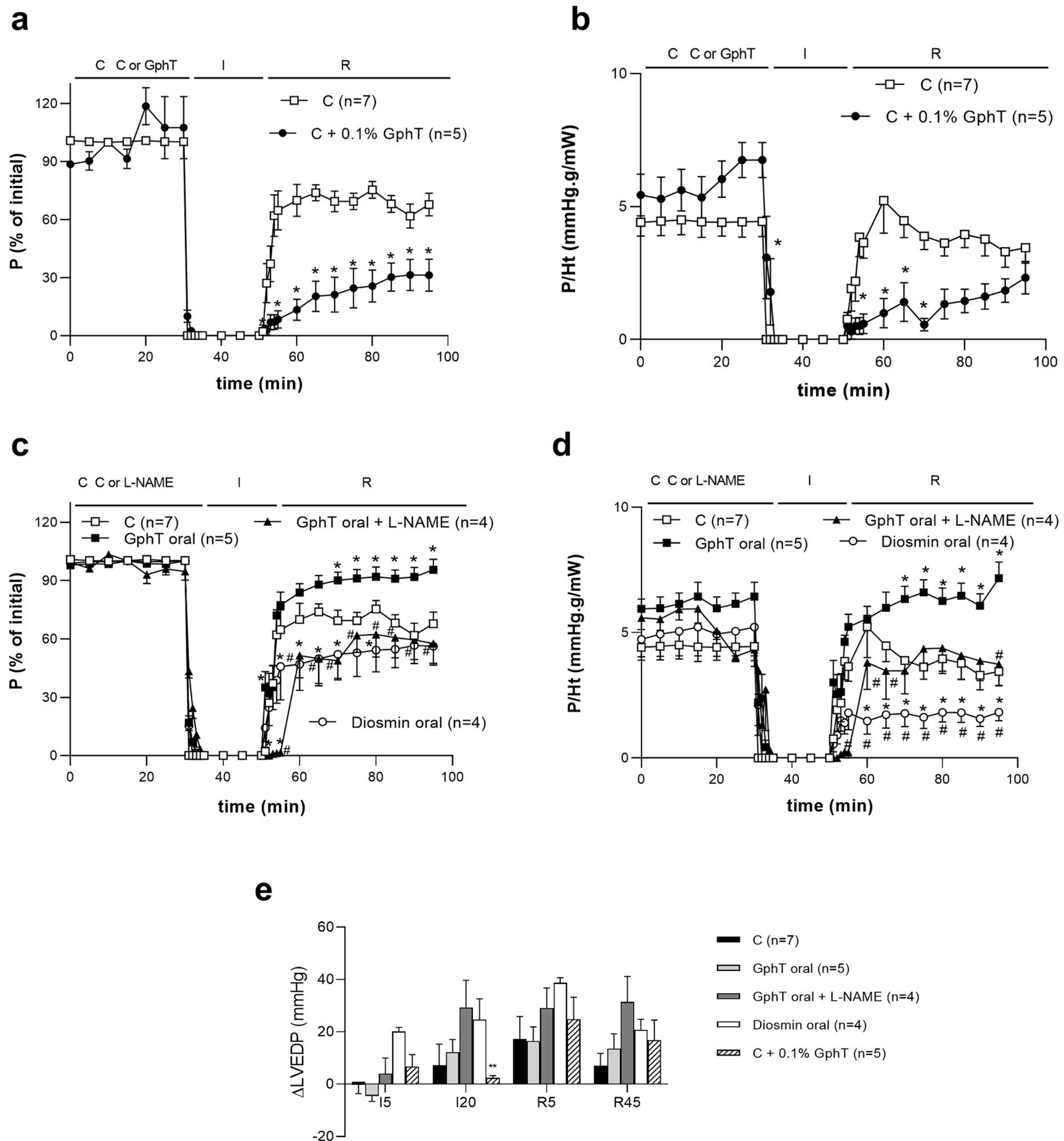


Fig. 4. Effect of perfusion of 0.1% v/v of *Gomphena perennis* L. tincture (GphT) and oral treatment of GphT or diosmin on (a,c) the contractile recovery (P, % of initial), (b,d) muscle economy (P/Ht) and (e) ΔLVEDP of rat hearts on I/R. Two-way ANOVA (Table A5). *A posteriori* Tukey's test: *p < 0.05 vs. control, #p < 0.05 vs GphT oral, **p < 0.05 vs GphT oral.

while eriocitrin showed antioxidant properties after oral administration.[35] Moreover, diosmin showed antihypertensive properties against DOCA-salt induced hypertension in rats.[36] Besides, different reports related the NO-dependent relaxant properties of flavonoids with their consequent vasodilator effects, mainly on resistance vessels and coronary arteries.[37] Specifically, isovitexin reduced the perfusion pressure in the mesenteric vascular beds of rats in a dose-dependent way, and this effect was reduced by the inhibition of NOS.[38] Since L-NAME did not prevent the decrease in SBP and DBP induced by GphT, these hypotensive effects did not involve NO release, discarding the participation of isovitexin. Since we previously demonstrated that GphT inhibited Ca^{2+} influx in the small intestine, this mechanism must be the main responsible for the hypotensive effect. On the other hand, GphT did not show diuretic properties in conscious rats at any dose of tincture included within the range in which hypotensive activity was demonstrated.

Considering the effect of GphT on BP and the reports showing that flavonoids prevented cardiac ischemia —probably via NO modulation[39] —, we evaluated the effects of GphT on isolated rat hearts. Perfusion of 0.1% w/v GphT induced a slight positive inotropic effect and increased muscle economy. However, this effect disappeared when the GphT concentration was increased. Since GphT showed calcium antagonist properties in rat small intestine, our findings could respond to this mechanism according to the cardiac effects of verapamil. In fact, verapamil improved cardiac efficiency and overall function, mainly because its potent vasodilator activity compensated its intrinsic negative inotropic effect.[40] However, perfusion of 0.1% GphT worsened the postischemic recovery of hearts exposed to I/R because it decreased muscle economy and contractile recovery with diastolic hypercontracture. Moreover, the effects of GphT in oral administration were evaluated on isolated hearts exposed to I/R, since this plant is orally

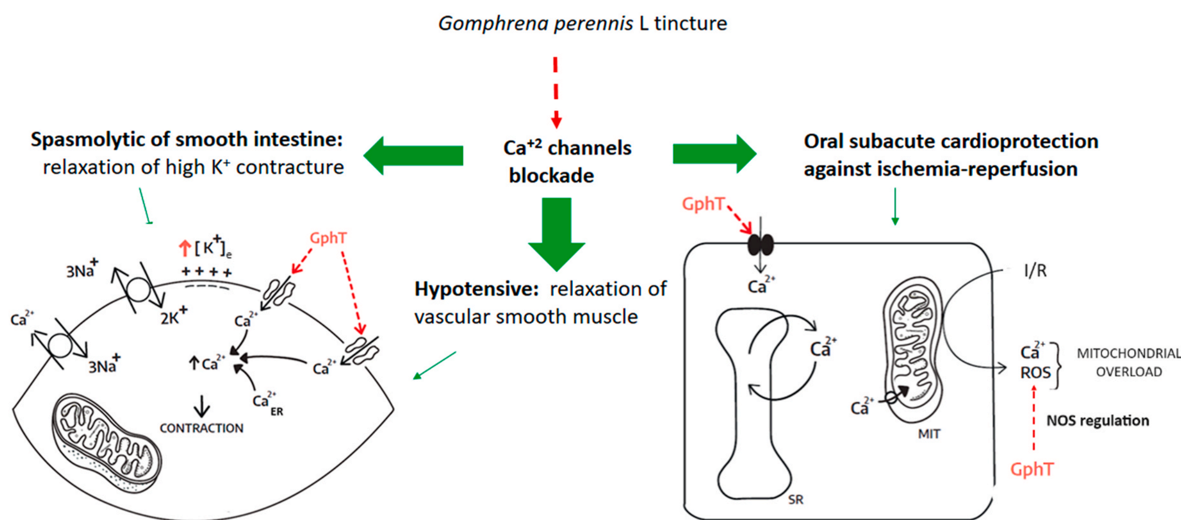


Fig. 5. Schematic diagram of the effect of *Gomphrena perennis* L. tincture (GphT) and its possible underlying mechanisms. ER: endoplasmic reticulum; SR: sarcoplasmic reticulum.

consumed by the population. This treatment prevented the dysfunction of postischemic hearts in a NO-release dependent way, as it was reversed by L-NAME. These results agreed with the reports about the capacity of flavonoids to reduce post-ischemic contractile dysfunction and infarct size, and to regulate the expression of iNOS and NOX2.[39,41,42] However, diosmin in oral administration did not improve cardiac I/R dysfunction. Although there were reports of its cardioprotective effects in ischemic hearts through a NOS-dependent mechanism, these beneficial effects occurred when diosmin was administered during reperfusion.[43] Besides, diosmin prevented oxidative stress and apoptosis, due to its antioxidant role in hearts exposed to longer ischemia.[44] Therefore, the presence of diosmin would not be responsible for the cardioprotective effects of oral GphT. Nevertheless, these beneficial effects could be at least partially related to the presence of eriocitrin and neoeriocitrin, since both components showed antioxidant properties. In fact, eriocitrin reduced the expression of inflammatory and cardiac biomarkers and myocardial malondialdehyde, and increased antioxidant enzymes and NO levels.[45] Moreover, neoeriocitrin, present in high amounts in extracts of bergamot pericarps, showed high free radical scavenging activity.[46]

Other chemical compounds such as saponins, tannins and steroids, as well as different types of flavonoids, were shown in *Gomphrena globosa* Linn. and *Gomphrena decumbens* extracts.[47] Therefore, future investigations will be necessary to identify other components of GphT and to determine which of them are responsible for its cardioprotective activity.

5. Conclusions

This work gives preclinical support to the potential use of *Gomphrena perennis* L. as an antispasmodic and hypotensive, and gives new knowledge about its cardioprotective effects against ischemic events (Fig. 5). The antispasmodic effect involves different mechanisms, mainly the non-competitive inhibition of Ca^{2+} influx. Although diuretic effects cannot be demonstrated in this work, our results show the hypotensive effect of GphT, which is not mediated by NO release but associated to the inhibition of Ca^{2+} influx. Contrarily, NO plays a crucial role in the cardioprotection induced by subacute oral treatment with GphT. These findings could be associated to the presence of flavones, characterized by RP-HPLC-DAD; however, further research is required to identify the compounds responsible for these actions.

In summary, the present study provides scientific evidence about ethnopharmacological uses of Gomphrena *perennis* L. which has not been previously assessed, and contributes to the knowledge for its use as a therapeutic alternative in patients with gastrointestinal spasms and/or cardiovascular diseases such as hypertension or angina pectoris.

Taxonomy (classification by EVISE)

Cardiovascular system – muscle - animals.
Experimental model system –
Traditional medicine.

Methodologies

High-performance liquid chromatography in a reverse phase oral administration, isolated perfused rat hearts, calorimetrical and mechanical measurements, isolated intestine rat, contractile concentration-response curves, intravenous administration.

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Declaration of competing interest

We have no known conflicts of interest in the publication of this article: Antispasmodic, cardioprotective and blood-pressure lowering properties of *Gomphrena perennis* L. and mechanisms of action.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2023.10.005.

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