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Research Paper

Vasorelaxant and hypotensive effects of the extract and the isolated flavonoid rutin obtained from *Polygala paniculata* L.

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Abstract

Objectives This study aimed to investigate the in-vitro and in-vivo cardiovascular effects of the crude hydroalcoholic extract from *Polygala paniculata* (HEPP) in rats.

Methods The procedures were performed on aortic rings and on normotensive anaesthetized rats.

Key findings When tested in endothelium-intact aorta rings, HEPP (30–1000 μg/ml) produced a significant non-concentration-dependent relaxing effect (~40%), which was completely prevented by incubation with L-NAME (nitric oxide synthase inhibitor), ODQ (soluble guanylate cyclase inhibitor) and partially inhibited by tetraethylammonium (TEA; a non-selective potassium channel blocker) and charybdotoxin (a large- and intermediate-conductance calcium-activated potassium channel blocker). In contrast, atropine (a muscarinic receptor antagonist) or pyrilamine(a histamine H_1 receptor antagonist) had no effect. Furthermore, oral administration of HEPP (30–300 mg/kg) in anaesthetized rats caused a dose-dependent and sustained hypotensive action. This effect was unchanged by atropine or TEA, but was strongly reduced in rats continuously infused with L-NAME or methylene blue. Moreover, rutin (1–3 mg/kg) administered by an intravenous route also caused a dose-dependent hypotensive effect in rats.

Conclusions Our results demonstrated that the extract obtained from *P. paniculata* induces potent hypotensive and vasorelaxant effects that are dependent on the nitric oxide/guanylate cyclase pathway. These effects could be related, at least in part, to the rutin contents in this extract.

Keywords hypotensive; nitric oxide; polygala paniculata; rutin; vasorelaxant

Introduction

The plants of the *Polygalaceae* family are widespread in tropical regions and are known to contain a great variety of biologically active compounds. The members of this family include the genus *Polygala*, with approximately 500 species.[1] *Polygala paniculata* Linneu is a native small bush that grows on the Brazilian Atlantic coast, known by the popular names of 'barba-de-são-joão', 'vassourinha branca' and 'mimosa'.[2] Furthermore, *P. paniculata* L. is used in folk medicine for the treatment of various inflammatory diseases, such as asthma, bronchitis, arthritis and other pathologies, including disorders of the kidney.[2,3]

Phytochemical studies carried out with plants of the genus *Polygala* revealed an abundance of several compounds, including cytotoxic lignans,[4] saponins,[5] xanthones, coumarins and flavonoids.[6] More recently, the isolation of three xanthones has been reported (1-hydroxy-5-methoxy-2,3-methylenedioxyxanthone, 1,5-dihydroxy-2,3-dimethoxyxanthone and 1-hydroxy-2.3.5-trimethoxyxanthone) together with coumarin murragatin, the flavonol rutin and two sterols, spinasterol and delta25-spinasterol from *P. paniculata* L.[7]

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Recently, we demonstrated the antinociceptive effect of the hydroalcoholic extract of *P. paniculata* (HEPP) and its isolated flavonoid rutin in chemical and thermal behavioral models of pain in mice.[8] Our group has also demonstrated that the HEPP exerts gastroprotective effects, probably involving prostaglandins and cytoprotective factors, such as maintenance of mucus production and antioxidant activity.[9] The antioxidant activity of HEPP was also related to the protective effects of this extract against methylmercury-induced neurotoxicity in mice.[10]

In addition, studies with plants of the genus *Polygala* have demonstrated activity in smooth muscle preparations *in vitro*, using rat aorta rings and guinea-pig trachea rings.[11,12] In agreement with this, the 1,7-dihydroxy-2,3-dimethoxy xanthone isolated from the hydroalcoholic extract of *P. cyparissias* caused a concentration-dependent relaxation in the trachea contracted by inflammatory mediators and in ovalbumin-actively sensitized trachea from the guinea-pig.[12] Furthermore, it has been shown that xanthones isolated from the roots of *P. caudata* exhibited scavenging and relaxing activity against the contractions evoked by KCl in Wistar rat thoracic aorta rings in a dose-dependent manner.[11]

Several studies have demonstrated that plant-derived extracts containing antioxidant compounds such as coumarins, xanthones and flavonoids have vasorelaxant and hypotensive effects both *in vitro* and *in vivo*, by acting in the vessels, probably through receptors expressed in the endothelial or smooth muscle cells.[11,13–18]

The vascular endothelium plays an important role in controlling vascular tone by the secretion of both relaxant and contractile factors. The endothelial cells respond to chemical and physical stimulation by producing relaxant factors such as endothelium-derived hyperpolarizing factor (EDHF), prostacyclin and nitric oxide.[19–22]

The aetiology of endothelium-dependent relaxation impairment is multi-factorial and the underlying mechanism is not yet fully elucidated. Among these factors, overproduction of reactive oxygen species under pathophysiological conditions, alterations of endothelial nitric oxide synthase (eNOS) expression and activity and decreased availability of NO, all have been considered responsible for the endothelial dysfunction.[23–25] After the release of NO from the endothelium, it diffuses into the arterial smooth muscle, activating guanylate cyclase[26] and thereby increasing cGMP formation, which leads to vasorelaxation with involvement of the potassium ion (K^+) channel opening in the vascular smooth muscle cell and closure of voltage-activated calcium (Ca^{+2}) channels.[27]

In addition, although some studies have demonstrated that the compounds found in the *Polygala species* have important pharmacological effects in the cardiovascular system, no pharmacological study has been carried out with *Polygala paniculata* evaluating its vascular effects. In the present investigation, we examined for the first time the possible action of the HEPP and its isolated flavonoid rutin 'in vitro' and 'in vivo' using either aorta rings or anesthetized normotensive animals, respectively. Moreover, the possible cellular mechanism involved in this effect was characterized.

Materials and Methods

Plant material

P. paniculata L. was collected at Daniela beach (Santa Catarina State, Brazil) and was classified by Olavo Araújo Guimarães (Universidade Federal do Paraná, Curitiba, Brazil). A voucher specimen was deposited in the herbarium of the Botany Department of the Universidade Federal do Paraná with the registration number UPCNB 26027.

Preparation of the hydroalcoholic extract and identification of its possible active compounds

The fresh whole plant was immersed in hexane three times (each time for 1 h) to remove the epicuticular wax. The plant without epicuticular wax was air-dried, powdered (3500 g of dry plant) and submitted to exhaustive extraction three times over one week by maceration with an 80 : 20 solution of ethanol : water. After maceration, the extract was filtered through a paper filter, and the solvent was evaporated under reduced pressure (50°C) in a rotary evaporator. Thus, the crude hydroalcoholic extract (yield 300 g) was obtained.

A portion of this crude extract (251 g) was subjected to a chain sequential chromatography fractionation. The fractions obtained were subjected to further purification procedures, such as flash chromatography, preparative TLC and recrystallization to yield 430 mg of the flavonoid quercetin-3-rutinoside (rutin), 192 mg of the phytosterol α-spinasterol and a set of five xanthones (1-hydroxy-5-methoxy-2,3-methylenedioxyxanthone (10 mg), 1,5-dihydroxy-2,3-dimethoxyxanthone (50 mg), 1,5-dihydroxy-6′,6′-dimethylpireno-(2′,3′:3,2)-xanthone (11 mg), 1-hydroxy-3,7-dimethoxyxanthone (8 mg) and 3,5-dihydroxy-6′,6′-dimethylpireno-(2′,3′:1,2)-xanthone (43 mg)). Rutin was the only flavonoid identified in the hydroalcoholic extract from *P. paniculata* (HEPP) and was found to be the major compound of the extract (0.17% of extract). The identification of all compounds was performed with various spectroscopic analyses (EIMS, IR, ^{1}H and ^{13}C NMR/DEPT, HMQC, HMBC and NOE).[7,28–30]

Pharmacological analysis

Animals

Male Wistar rats, 250–300 g, from the colony of the Federal University of Paraná were used in this study. The rats were kept on a constant 12-h light–dark cycle with controlled temperature (22 ± 2°C). Standard pellet food (Nuvital, Curitiba, PR, Brazil) and water were freely available. The institutional Ethics Committee of the Universidade Federal do Paraná approved all procedures adopted in this study (project number 264).

Drugs

Phenylephrine hydrochloride, acetylcholine chloride, N^{ω}-nitro-L-arginine-methyl-ester (L-NAME), 1H-[1,2,4] oxadiazolo[4,3-alpha]quinoxalin-1-one (ODQ), methylene blue, atropine, pyrilamine, tetraethylammonium, glibenclamide, 4-aminopyridine, apamine and charybdotoxin were purchased from Sigma (St Louis, MO, USA). All other reagents were of the highest grade. All other drugs and solutions were

prepared in fresh distilled water. Rutin, a flavonoid isolated from *P. paniculata*, was dissolved in DMSO to prepare a minimum volume of DMSO in an organ bath or for a blood flow study, which did not alter the normal response of tissues *per se*.

Measurement of vascular relaxation and investigation of mechanism involved

The rat aorta rings were prepared as previously described,[31] using organ baths containing 3 ml of Krebs–Henseleit buffer, pH 7.4 (composition in mM: NaCl 115.3, KCl 4.9, $CaCl_2.2H_2O$ 1.46, KH_2PO_4 1.2, $MgSO_4$ 1.2, D-glucose 11.1, $NaHCO_3$ 25). The ability of HEPP to cause vascular relaxation was studied in both endothelium-denuded and endothelium-intact aorta rings previously contracted by phenylephrine (PHE, 1 μM). Under the sustained contraction elicited by PHE, the vessels were exposed to cumulative concentrations of HEPP (30–1000 μg/ml). In a separate set of experiments using only endothelium-intact rings, this same procedure was performed in preparations subjected to 15 min incubation in the presence of L-NAME (10 μM; a non-selective nitric oxide synthase inhibitor), ODQ (10 μM; a soluble guanylate cyclase inhibitor), tetraethylammonium (TEA, 10 mM; a non-selective potassium channel blocker), glibenclamide (GLB, 10 μM; an ATP-sensitive potassium channel blocker), 4-aminopyridine (4-AP, 1 mM; a voltage-sensitive potassium channel blocker), apamine (0.1 μM; a small conductance calcium-activated potassium channel blocker), charybdotoxin (0.1 μM; a large and intermediate-conductance calcium-activated potassium channel blocker), atropine (1 μM, a muscarinic receptor antagonist) or pyrilamine (10 μM, a histamine H1 receptor antagonist). Finally, to address any residual or non-reversible effects of HEPP on contractile or relaxation events, aorta rings (exposed to cumulative concentrations of HEPP) were washed and allowed to recover for 60 min before additional exposure to PHE (1 μM) and acetylcholine (1 μM).

Direct blood pressure measurements in anaesthetized rats

Male Wistar rats, 250–300 g, were anaesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg), given by the intramuscular route. A polyethylene catheter was inserted into the right femoral vein for administration of the drugs. Immediately after obtaining venous access, a bolus injection of heparin (300 IU) was administered intravenously. The rats were allowed to breathe spontaneously through a tracheotomy. The left carotid artery was cannulated and connected to a pressure transducer coupled to a MacLab recording system (MacLab/8), allowing the recording of the mean arterial pressure (MAP). For stabilization of blood pressure after the surgical procedures, a resting period of 15 min was imposed before the injection of any drug.

Administration of hydroalcoholic extract from P. paniculata *and its isolated flavonoid rutin*

One hour before the measurement of blood pressure, different groups of rats received HEPP by the oral route at doses of 30, 100 and 300 mg/kg. This first set of experiments was performed to evaluate the HEPP hypotensive effects. After this, a dose of 100 mg/kg of HEPP was chosen to study the time course effects of HEPP and the mechanisms involved in hypotensive effects. In the second set of experiments, the HEPP (100 mg/kg, p.o.) was administered, and the MAP was measured at different time points (30–180 min) to study the time course effects of the extract.

To evaluate the hypotensive effects of rutin, the isolated compound rutin was given intravenously at doses of 1, 2 and 3 mg/kg, 15 min after surgery, to permit stabilization of MAP. Acetylcholine (30 nmol/kg, i.v.) was used as the reference drug.

Treatment with muscarinic receptor antagonist

In these experiments, a single dose of atropine (5 mg/kg, s.c.) was administered to the rats 15 min before the administration of HEPP (100 mg/kg, p.o.), and the arterial blood pressure was measured as described above. The hypotension induced by HEPP was compared with the results obtained in the control rat that did not receive the antagonist and was only treated with HEPP (100 mg/kg, p.o.).

Infusion of L-NAME or methylene blue

In this set of experiments, the rats pre-treated with HEPP (100 mg/kg, p.o., for 1 h) were prepared for MAP recording and had both the left and right femoral veins cannulated. The right femoral vein was used for bolus administration of heparin 30 IU, while the contralateral vein was connected to an infusion pump (EFF 311; Insight, Ribeirão Preto, Brazil). Different groups of rats received a continuous infusion of L-NAME (7 mg/kg per min) or methylene blue (150 nmol/kg per min) over 20–30 min, and the ability of HEPP to reduce the MAP under the continuous infusion of L-NAME or methylene blue was verified. The total volume injected into the rats during the infusion period was 900 μl.

Treatment with potassium channel blocker

The effect of HEPP (100 mg/kg, p.o.) was measured before and 10 min after treatment with tetraethylammonium (360 μmol/kg, i.v.), a non-selective potassium channel blocker), and the control group received only the vehicle (p.o.). The dose of potassium channel blocker was based on previous studies.[32]

Statistical analysis

The data are expressed as mean ± SEM of the results of the 5–8 experiments. Differences across the groups were determined with one-way analysis of variance followed by Newman–Keuls' tests or *t*-tests subjected to Bonferroni's correction, when necessary. $P < 0.05$ was considered statistically significant. Generation of graphs and statistical analysis were performed using the GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Vasorelaxant effects of hydroalcoholic extract from *P. paniculata*

To study the vasorelaxant activity of the preparation, rat thoracic aorta rings with and without endothelium were

pre-contracted with phenylephrine (1 μM), and the effects of cumulative concentrations of the HEPP (30–1000 μg/ml) were observed. The addition of cumulative concentrations of HEPP produced a significant relaxation (39.4 ± 3.8%) that was detected at the dose of 1000 μg/ml only in the endothelium-intact preparation (Figure 1a). Thus, the findings suggest that the vasorelaxant effect of the extract on aortic rings was endothelium dependent.

The HEPP-induced relaxation was completely prevented by the non-selective nitric oxide synthase inhibitor L-NAME (Figure 1b) and the soluble guanylate cyclase inhibitor ODQ (Figure 1c). In addition, incubation with atropine (a muscarinic receptor antagonist) was found to have no effect in our experiments, while incubation with the non-selective potassium channel blocker, tetraethylammonium (TEA), reduced the relaxation induced by HEPP by approximately 50.0 ± 17% (Figure 2a and 2b).

The pre-incubation of the aorta rings with charybdotoxin, but not with glibenclamide, 4-aminopyridine, apamine or pyrilamine, also prevented the vasorelaxant effect of HEPP (results not shown).

Hypotensive effects of hydroalcoholic extract from *P. paniculata* in normotensive rats

The basal MAP was recorded in anaesthetized rats for 15 min, which corresponded to the period allowed for MAP stabilization. Before the administration of any drug, the recorded MAP was 105.5 ± 1.6 mmHg. The oral administration of HEPP at doses of 30, 100 and 300 mg/kg caused a significant dose-dependent reduction in the blood pressure (15.0 ± 1.3, 22.5 ± 1.6, 27.0 ± 1.5 mmHg, respectively), when compared with basal MAP (Table 1). The dose of 100 mg/kg was chosen due the limited amount of the extract and because this dose had reduced the MAP in a similar manner to the dose of 300 mg/kg. A significant effect of HEPP (100 mg/kg, p.o.) on arterial pressure was observed at 30, 60, 120 and 150 min after oral administration, with a maximal hypotensive effect at 60 min (22.0 ± 3.1) when compared with the basal group (Table 1).

The involvement of the nitric oxide-guanylate cyclase pathway in the hypotensive effects of hydroalcoholic extract from *P. paniculata*

The continuous infusion of L-NAME (7 mg/kg per min, a non-selective nitric oxide synthase inhibitor) raised the MAP from 103.0 ± 2.2 mmHg ($n = 6$) to 167.9 ± 4.7 mmHg. With L-NAME infusion, the ability of HEPP (100 mg/kg, p.o.) to reduce MAP was strongly inhibited (77 ± 13%) (Table 2). On the other hand, infusion of methylene blue (150 nmol/kg per min; a soluble guanylate cyclase inhibitor) did not cause any significant changes in the MAP.

Lack of involvement of cholinergic and potassium channel pathways

The hypotensive effect of HEPP (100 mg/kg, p.o.) was not reduced in rats previously treated with atropine (5 mg/kg; a muscarinic receptor antagonist) or TEA (360 μmol/kg; potassium channel blocker). The doses of atropine and TEA used did not change the basal MAP (Table 2).

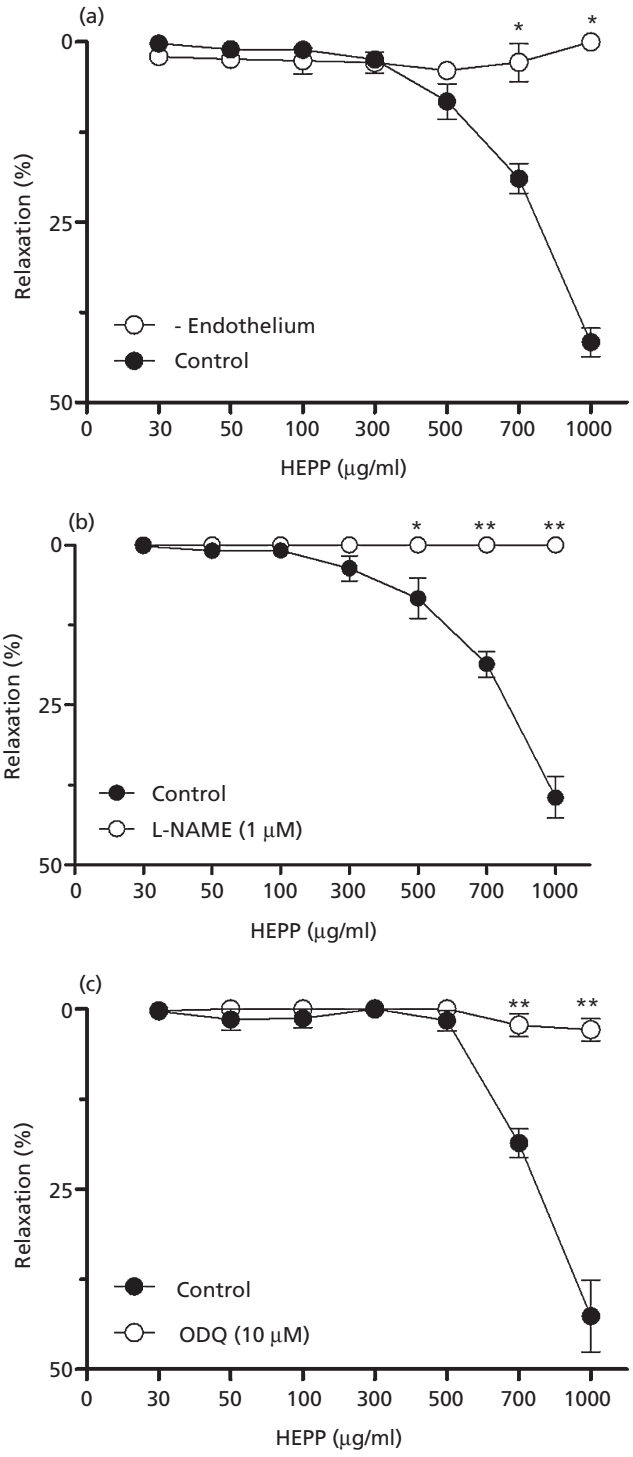

Figure 1 Endothelium- and nitric oxide/cGMP- dependent relaxation induced by HEPP in rat aorta rings. (a) Relaxing activity of HEPP (30, 50, 100, 300, 500, 700, 1000 μg/ml) in endothelium-intact (indicated by the label 'Control') and endothelium-denuded (indicated by the label 'Endothelium') aorta. (b) Influence of L-NAME (1 μM), a nitric oxide synthase inhibitor, and (C) ODQ (10 μM), a guanylate cyclase inhibitor, on the vasorelaxant effect of HEPP. The results represent the mean of the values obtained in 5–7 experiments, and the error bars indicate the SEM. *$P < 0.05$; **$P < 0.01$ compared with the respective control group (one-way analysis of variance followed by Newman–Keuls test).

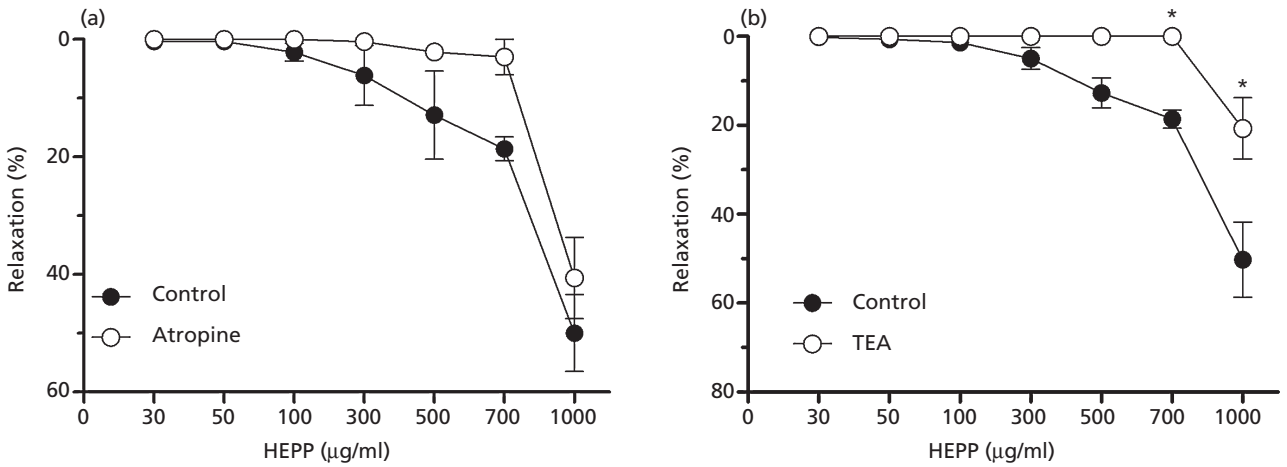

Figure 2 Relaxing effects of HEPP in the presence of muscarinic antagonist and potassium channels blocker. Activity of HEPP in the absence (control curves) or in the presence of the (a) atropine (1 μM; a muscarinic receptor antagonist) or (b) tetraethylammonium (TEA; 10 mM; a non-selective potassium channel blocker). The results represent the mean of the values obtained in 5–7 experiments, and the error bars indicate the SEM. $^{*}P < 0.05$ compared with the respective control group (one-way analysis of variance followed by Newman–Keuls test).

Table 1 Hypotensive effect of HEPP, acetylcholine and rutin on anaesthetized rats

Treatment	Doses	Change in MAP (mmHg)
Basal value	–	4.8 ± 1.0
HEPP (p.o.)	30 mg/kg	15,0 ± 1.3*
	100 mg/kg	22.5 ± 1.6**
	300 mg/kg	27.0 ± 1.5**
Basal value	–	3.5 ± 0.7
Acetylcholine (i.v.)	30 nmol/kg	42.9 ± 4.7***
Rutin (i.v.)	1 mg/kg	14.2 ± 1.8*
	2 mg/kg	21.5 ± 1.7**
	3 mg/kg	29.4 ± 2.8***
Treatment	**Time (min)**	**Change in MAP (mmHg)**
Basal value	–	3.5 ± 0,7
HEPP	30	10.2 ± 2.2*
(100 mg/kg, p.o.)	60	22.0 ± 3.1**
	120	13.5 ± 1.7*
	150	12.0 ± 2.4*
	180	2.0 ± 0.6

The results show the mean ± SEM, $n = 6$. $^{*}P < 0.05$; $^{**}P < 0.01$ or $^{***}P < 0.001$ compared with control (one-way analysis of variance followed by *t*-test subjected to Bonferroni's correction)

Table 2 Effects of L-NAME, methylene blue, atropine or TEA on hypotensive effect of HEPP in rats

Treatment	Doses	Change in MAP (mmHg)
Saline	–	3.9 ± 0.6
HEPP (p.o.)	100 mg/kg	21.7 ± 1.7***
L- NAME	7 mg/kg per min.	22.2 ± 2.7***
HEPP (p.o.) + L- NAME	100 mg/kg + 7 mg/kg per min.	5 ± 2.8$^{\#\#\#}$
Methylene blue	150 nmol/kg per min.	24.0 ± 2.8***
HEPP (p.o.) + Methylene blue	100 mg/kg + 150 nmol/kg per min.	2.5 ± 1.2$^{\#\#\#}$
Saline	–	4.8 ± 0.7
HEPP (p.o.)	100 mg/kg	16.9 ± 1.6***
Atropine (s.c.)	5 mg/kg	3.6 ± 0.8
HEPP (p.o.) + Atropine (s.c.)	100 mg/kg + 5 mg/kg	24.9 ± 1.8***
TEA (i.v.)	360 μmol/kg	3.6 ± 0.8
HEPP (p.o.) + TEA (i.v.)	100 mg/kg + 360 μmol/kg	20.0 ± 3.1***

The results show the mean ± SEM, $n = 6$. $^{***}P < 0.001$ compared with the saline group; $^{\#\#\#}P < 0.001$ compared with the HEPP-treated group (one-way analysis of variance followed by *t*-test subjected to Bonferroni's correction)

Hypotensive effects of rutin in normotensive rats

The intravenous administration of rutin at doses of 1, 2 and 3 mg/kg caused a significant dose-dependent reduction in blood pressure (Table 1) with a maximal hypotensive effect of 29.4 ± 2.8 mmHg. Acetylcholine at 30 nmol/kg (reference drug) significantly reduced the MAP (42.9 ± 4.7 mmHg).

Discussion

The results demonstrated that the HEPP induced non-concentration-dependent relaxation in aortic rings pre-contracted by phenylephrine. Furthermore, the oral administration of the hydroalcoholic extract from whole parts of *P. paniculata* (HEPP) and the intravenous administration of rutin resulted in a dose-dependent and reversible reduction in the mean arterial pressure of anaesthetized rats.

Recent studies have shown that some species of *Polygala* were able to relax smooth muscle preparations *in vitro*. A preliminary study showed that the xanthones isolated from the roots of *P. caudata* exhibited relaxing activity on the contractions induced by KCl in rat thoracic aorta rings in a dose-dependent manner.[11] In addition, another study showed that an isolated xanthone (dihydroxy-2.3-dimethoxyxanthone) from *P. cysparissias* presented relaxant effects in rat tracheal smooth muscle.[12]

Our study demonstrated that *P. paniculata* exerted an important vasorelaxant effect both *in vivo* and *in vitro*. Phytochemical studies have revealed that the major compound identified in HEPP was the flavonoid rutin; compounds such as xanthones were identified but were minor constituents.[7] This study assessed the effects of the HEPP in the cardiovascular system because a large number of flavonoids found in plant derived-extracts have been shown to have strong potential for the treatment of cardiovascular diseases.[33–36]

In this work, the vasorelaxant action of HEPP was tested in aortic rings with endothelium and in denuded aortic rings. The effect of the HEPP seemed to be endothelium dependent, as the vasorelaxant action of HEPP was abolished in denuded aortic rings. The mechanism underlying the endothelium-dependent relaxation by HEPP was confirmed with the use of the nitric oxide synthase inhibitor L-NAME (10 μM), which markedly reduced the HEPP-induced relaxation of endothelium-intact aortas. Moreover, the infusion of L-NAME (7 mg/kg per min) *in vivo* also reduced the hypotension caused by HEPP. The involvement of cGMP in the relaxant and hypotensive effects of HEPP was verified *in vitro*, using a soluble guanylate cyclase inhibitor, ODQ, and *in vivo*, using a methylene blue (150 nmol/kg per min) infusion. These compounds markedly antagonized the HEPP-induced relaxation in the isolated aorta and the hypotension in normotensive rats. These findings clearly show that the aortic relaxation and hypotension caused by HEPP is mediated by both the endothelium and vascular smooth muscle via NO production/release and a cGMP-dependent mechanism.

In addition, various agonists stimulate NO release from endothelial cells, including acetylcholine and histamine, through interactions with specific endothelial receptors.[37] Thus, we investigated the effects of atropine and pyrilamine on HEPP-induced relaxation. Our results demonstrated that these two antagonists were unable to inhibit the vasorelaxation or hypotensive actions of HEPP, indicating that muscarinic and histaminic receptors probably were not involved in the HEPP effect.

Following these findings, additional experiments were carried out with the aim to investigate whether the flavonoid rutin isolated from HEPP was able to reduce the mean arterial pressure of anaesthetized rats. Our results showed that the intravenous treatment with rutin in the range of doses tested produced a reversible hypotension. These data corroborate studies that have shown that rutin is a flavonoid with vasoactive effects.[16,17] Thus, these data strongly suggest that rutin contributes to the vasorelaxant effects of the HEPP. However, one cannot exclude the possibility that other compounds (e.g. flavonoids and chalcones among others) might contribute individually or synergistically to this effect of HEPP *in vitro* and *in vivo*.

Moreover, some studies have shown that orally administered rutin can be converted to aglycon or glucosilate/glycosilate metabolites that have vasodilatory effects.[16,38,39] In addition, the effect observed with intravenous administration of rutin might be related to its antioxidant activity. Recently, some studies have shown that several flavonoids produced a concentration-dependent relaxation in rat isolated aortic rings, an effect that seemed to be related to antioxidant activity.[25,40,41] Moreover, a study conducted by our group has demonstrated the antioxidant action of the HEPP and its flavonoid rutin in the DPPH assay.[9] Thus, these data led us to hypothesize that the antioxidant activity of the extract given orally or the flavonoid given intravenously could be one of the mechanisms involved in the vasodilatory activity caused by the increase in nitric oxide bioavailability. However, additional studies are necessary to confirm this hypothesis.

This work demonstrated that the vascular relaxation caused by HEPP involves the activation of calcium-activated potassium channels of intermediate- and large- conductance, as the pre-incubation with tetraethylammonium (Figure 2b) or charybdotoxin (results not shown) partially reversed the aorta relaxation elicited by HEPP, although the hypotensive effects induced by HEPP were not altered by treatment *in vivo* with tetraethylammonium (360 μmol/kg, i.v.). The inability of tetraethylammonium to alter the effects of the HEPP *in vivo* suggests that potassium channels are more important for HEPP *in vitro* than *in vivo*. This could be explained by the network of pressure control systems in the body, which involves not only endothelial factors, but also several neural receptors, hormonal controllers (e.g. angiotensin, vasopressin, aldosterone), the pumping ability of the heart and the kidney pressure control system,[42] which are several mechanisms where the potassium channels are not decisive targets for achieving this final effect.

The participation of small-conductance calcium-activated potassium channels and ATP-sensitive potassium channels was also investigated; however, the aorta relaxation induced by HEPP was not changed after incubation with apamine, a small-conductance calcium-activated potassium channel blocker or in the presence of glibenclamide, a selective blocker of ATP-dependent potassium channels (results not shown). Our pharmacological findings have highlighted the hypotensive and vasorelaxant effects of *P. paniculata* in the cardiovascular system and suggest a mechanism that involves enhancement of the NO-cGMP system with activation of calcium-activated potassium channels.

Conclusions

Our study demonstrated that the crude hydroalcoholic extract from *Polygala paniculata* induces vascular relaxation in rat aortic rings, an effect confirmed by the hypotensive effects in anaesthetized rats. These effects appear to be dependent on the stimulation of the activation of the nitric oxide/guanylate cyclase pathway and subsequent opening of calcium-activated potassium channels. The development of pharmacological strategies that are able to stimulate or maintain endogenous nitric oxide production may contribute to improved management of several pathological conditions, such as hypertension and atherosclerosis. Together, our findings may contribute to providing the pharmacological basis for the use of *Polygala paniculata* in folk medicine and have demonstrated its potential as a phytomedicine.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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