Blood pressure reducing effects of Phalaris canariensis in normotensive and spontaneously hypertensive rats

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Blood pressure reducing effects of *Phalaris canariensis* in normotensive and spontaneously hypertensive rats

Clévia Santos Passos, Lucimeire Nova Carvalho, Roberto Braz Pontes, Jr., Ruy Ribeiro Campos, Olinda Ikuta, and Mirian Aparecida Boim

Abstract: The birdseed *Phalaris canariensis* (Pc) is popularly used as an antihypertensive agent. The aqueous extract of Pc (AEPc) was administered in adult normotensive Wistar rats and spontaneously hypertensive rats (SHR) and in prehypertensive young SHR (SHR_Y, 3 weeks old). Animals received AEPc (400 $mg \cdot kg^{-1} \cdot day^{-1}$, by gavage) for 30 days, then groups were divided into 2 subgroups: one was treated for another 30 days and the other received water instead of AEPc for 30 days. AEPc reduced systolic blood pressure (SBP) in both adult groups; however, treatment interruption was followed by a gradual return of the SBP to baseline levels. SHR_Y became hypertensive 30 days after weaning. AEPc minimized the increase in SBP in SHR_Y, but blood pressure rose to levels similar to those in the untreated group with treatment interruption. There were no changes in renal function, diuresis, or Na^+ excretion. Pc is rich in tryptophan, and the inhibition of the metabolism of tryptophan to kynurenine, a potential vasodilator factor, prevented the blood pressure reducing effect of AEPc. Moreover, AEPc significantly reduced sympathoexcitation. Data indicate that the metabolic derivative of tryptophan, kinurenine, may be a mediator of the volume-independent antihypertensive effect of Pc, which was at least in part mediated by suppression of the sympathetic tonus.

Key words: hypertension, *Phalaris canariensis*, renal function, tryptophan, kynurenine, SHR, sympathetic tone.

Résumé : Le *Phalaris canariensis* (Pc) est couramment utilisé comme antihypertenseur. On a administré un extrait aqueux de Pc (EAPc) à des rats Wistar adultes normotendus et spontanément hypertendus (RSH) ainsi qu'à de jeunes RSH pré-hypertendus (RSHj, âgés de 3 semaines). On a administré l'EAPc (400 mg/kg/j, par gavage) au rats pendant 30 jours, puis on a divisé ces rats en 2 sous-groupes : l'un a été traité à l'EAPc pendant 30 jours supplémentaires, l'autre a ingéré de l'eau à la place de l'EAPc pendant 30 jours. L'EAPc a réduit la pression artérielle systolique (PAS) chez les deux groupes adultes; toutefois, l'interruption du traitement a été suivie d'un retour graduel de la PAS aux valeurs de base. Les RSHj sont devenu hypertendus 30 jours après le sevrage. L'EAPc a réduit l'augmentation de la PAS chez les RSHj; cependant, après l'interruption du traitement, la PAS a augmenté aux mêmes valeurs que celles du groupe non traité. La fonction rénale, la diurèse ou l'excrétion de Na^+ sont demeurées stables. Le Pc est riche en tryptophane, et l'inhibition du métabolisme du tryptophane en kynurénine, un facteur potentiel de vasodilatation, a prévenu les effets abaisseurs de pression artérielle de l'EAPc. De plus, l'EAPc a réduit significativement la sympatho-excitation. Les résultats indiquent que le dérivé métabolique du tryptophane, kinurénine, pourrait être un médiateur de l'effet antihypertenseur, indépendant du volume, du Pc, effet qui a été en partie véhiculé par la suppression du tonus sympathique.

Mots-clés : hypertension, *Phalaris canariensis*, fonction rénale, tryptophane, kynurénine, RSH, tonus sympathique.

[Traduit par la Rédaction]

Introduction

The global prevalence of hypertension is approximately 1 billion individuals (Cutler et al. 2008), and it causes almost 6% of deaths worldwide each year, of which 40% are due to stroke (Chobanian et al. 2003) and 25% are due to coronary heart disease (Chobanian et al. 2003; Kearney et al. 2005). In addition to the cardio-cerebrovascular injury, hypertension appears to be a significant risk factor for developing renal failure (National Institutes of Health 1997; Doggrell and Brown 1998; Chobanian et al. 2003).

Strategies for the treatment of hypertension are used most often in an integrated way (pharmacological and nonpharmacological treatments, including physical exercise, control of sodium intake, etc.). Herbal medicines are widely used for therapeutic purposes in several countries (Turolla and Nascimento 2006). The World Health Organization recommends

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phytotherapy as a way to reduce the costs of public health programs, suggesting promotion of the dissemination of knowledge required for its use (World Health Organization 2004).

The seed of *Phalaris canariensis* L. (Pc), or canary seed, is a member of a family of grasses (Graminaceae), and it is used in folk medicine in the form of tea as a co-adjuvant in the treatment of hypertension, diabetes mellitus, and hypercholesterolemia (Novas et al. 2004), with or without other forms of traditional therapy (Merzouki et al. 2003); however, such use has no scientific basis. There is only one study related to the hypotensive effect of Pc seed infusion in normotensive rats (Balbi et al. 2008); however, its therapeutic use as an antihypertensive agent and its possible mechanisms of action have not been scientifically demonstrated.

The aim of the present study was to establish the effect and dosage of the aqueous extract of *P. canariensis* (AEPc) and to determine whether it was able to acutely reduce arterial blood pressure in awake normotensive or spontaneously hypertensive rats (SHR). Additionally, we evaluated the systemic and renal effects of chronic administration of AEPc in both groups. Finally, we analyzed whether AEPc administration soon after weaning, i.e., during the prehypertension stage in young SHR, would be able to prevent, minimize, or delay the onset of hypertension. Pc is an important source of tryptophan (Abdel-Aal et al. 1997), and it has been recently shown that the metabolism of tryptophan to kynurenine by the indoleamine 2,3-dioxygenase (IDO) pathway contributes to vessel relaxation and reduces blood pressure in SHR (Wang et al. 2010). The possible role of kynurenine as a mediator of the hypotensive effect of AEPc was evaluated in adult SHR receiving an IDO inhibitor.

Material and methods

Adult 3-month-old male Wistar rats, spontaneously hypertensive rats (SHR), and normotensive young SHR (SHR_Y, 3 weeks old) were purchased from the Center for the Development of Experimental Models for Medicine and Biology (Federal University of São Paulo). The animals were housed in a temperature-controlled environment and grouped in collective cages ($n = 5$) with a light–dark cycle (12 h light : 12 h dark). They received standard rat chow (Nuvital) and tap water ad libitum. This study was approved by the ethics committee of the Federal University of São Paulo (1326/08).

Aqueous extraction of *Phalaris canariensis* seeds

Phalaris canariensis. seeds were purchased from Beppler Imp. & Exp. Ltda (São Paulo, Brazil). The aqueous extract (10%) was prepared using 100 g of crushed seeds and 1 L of distilled water. The mixture was kept at 70 °C for 30 min with periodic stirring every 10 min. After filtration through a cloth strainer, the infusion was concentrated to one-fifth of its initial volume in a rotary vacuum evaporator (Fisatom, model 802, São Paulo, Brazil) at 50 °C. The aqueous extract was lyophilized in a freeze dryer (Heto, model FD8, Allerod, Denmark) and stored in plastic bottles.

Amino acid profile of the aqueous extract of *Phalaris canariensis*

A 100 mg sample of AEPc was diluted in 1 mL of distilled water and centrifuged for 1 h at 4° C. The sample was evaporated in liquid nitrogen, resuspended in 500 μL of 25% acetonitrile, and then injected into the high-performance liquid chromatography column (Shimadzu SCL-10A VP system, Shimadzu Corporation, Tokyo, Japan).

Fig. 1. Dose-dependent effects of the aqueous extract of *Phalaris canariensis* on mean arterial pressure (MAP) in awake spontaneously hypertensive rats (mean of 3 animals per dose). Data are expressed as the mean ± SE.

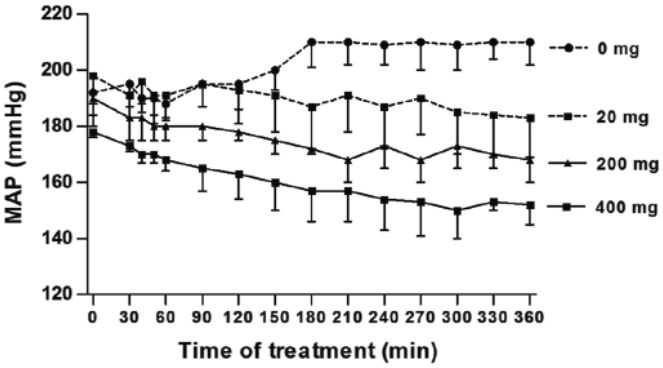
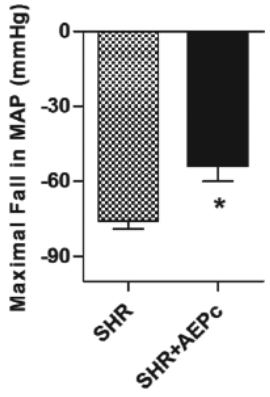


Fig. 2. Maximal fall in mean arterial pressure (MAP) in response to acute administrations of hexamethonium bromide (30 mg·(kg body mass)$^{-1}$, intravenous injection) in spontaneously hypertensive rats (SHR) or SHR treated with aqueous extract of *Phalaris canariensis* (SHR + AEPc). *, $p < 0.05$.



Experimental design: acute effects of the aqueous extract of *Phalaris canariensis*

The dosage of AEPc was previously determined in a previous series of experiments by observing the acute effect of increasing doses on the blood pressure (BP) in catheterized awake adult SHR for periods of up to 6 h. Twenty-four hours after femoral artery cannulation, the pulsatile and mean arterial BP were recorded in conscious animals by an analog–digital board (PowerLab, ADInstruments, Bella Vista, Australia) as previously described (Oliveira-Sales et al. 2008). Before use, the extract was dissolved in 1 mL of distilled water at concentrations of 20, 200, and 400 mg·kg^{-1} and administered by gavage. The control group received only 1 mL of distilled H_2O. Animals were subjected to a baseline recording of BP for 30 min before AEPc administration. As shown in Fig. 1, all doses reduced the BP, but the dose of 400 mg·kg^{-1} (per rat) was the most effective; thus, it was used in the chronic experiments.

Table 1. Body mass, water intake, and food intake of adult male Wistar (W) rats, spontaneously hypertensive rats (SHR), and prehypertensive young SHR (SHR_Y).

	Body mass (g)			Water intake (mL·24 h $^{-1}$)			Food intake (g·24 h^{-1})		
Time	W	SHR	SHR $_Y$	W	SHR	SHR_Y	W	SHR	SHR_Y
H_2O									
Day 0	279±10	222±8	70±3	34±3	29±4	9±1	21±2	20±2	13±1
Day 30	391±9a	281±17a	178±9a	44±4a	36±3	34±2a	21±2	20±2	19±1
Day 60	424±8ab	323±18a	242±5ab	42±4	35±3	31±2a	20±1	21±1	24±1
Pc									
Day 0	288±8	245±11	62±4	30±3	31±1	9±1	19±1	20±2	12±1
Day 30	432±12a	290±5a	182±5a	30±3	34±2	28±4a	19±1	23±1	21±1
Day 60	473±11ab	307±7a	254±8ab	33±3 ab	34±4	30±1a	18±3	22±1	27±2
Pc-H_2O									
Day 0	288±8	241±9	59±5	30±3	31±4	10±3	19±1	22±2	13±1
Day 30	432±12a	287±4a	176±5a	30±3	34±2	29±2a	19±1	23±2	23±2
Day 60	459±27	303±8a	257±3ab	21±1	30±5	37±3a	15±2	24±1	31±1

Note: Values are expressed as the mean ± SE (n = 5 rats for each group). Groups received tap water (H_2O) for 60 days, aqueous extract of *Phalaris canariensis* (Pc) for 60 days, or treatment interruption after 30 days followed with tap water for another 30 days (Pc-H_2O). Values followed by different letters are significant at $p \leq 0.05$; a, significant compared with day 0; b, compared with day 30 of the respective group.

Table 2. Plasma creatinine (Cr), sodium (Na^+), and potassium (K^+) levels in adult male Wistar (W) rats, spontaneously hypertensive rats (SHR), and prehypertensive young SHR (SHR_Y).

	Cr (mg·dL^{-1})			Na^+ (mequiv·L^{-1})			K^+ (mequiv·L^{-1})		
Time	W	SHR	SHR_Y	W	SHR	SHR_Y	W	SHR	SHR_Y
H_2O									
Day 0	0.6±0.1	0.5±0.1	—	142±1	141±1	—	5.2±0.2	5.0±0.1	—
Day 30	0.6±0.1	0.5±0.1	0.5±0.1	143±1	150±5	141±1	5.0±0.1	5.0±0.1	5.0±1
Day 60	0.7±0.1	0.6±0.1	0.7±0.1	137±1	140±1	131±7	5.0±0.1	5.0±0.1	5.5±1
Pc									
Day 0	0.5±0.1	0.5±0.1	—	148±3	147±6	—	5.4±0.2	5.0±0.1	—
Day 30	0.5±0.1	0.5±0.1	0.5±0.1	141±1	138±4	140±1	4.2±0.2[a]	5.0±0.1	5.0±1
Day 60	0.6±0.1	0.6±0.1	0.6±0.1	141±4	141±2	141±1	5.6±0.1[b]	5.0±0.1	4.8±1
Pc-H_2O									
Day 0	0.5±0.1	0.4±0.1	—	148±3	142±4	—	5.4±0.2	5.0±0.1	—
Day 30	0.5±0.1	0.6±0.1	0.5±0.1	141±1	144±8	141±1	4.2±0.2	5.5±0.1	5.5±1
Day 60	0.5±0.1	0.5±0.1	0.5±0.1	145±1	140±2	140±1	5.5±0.5	5.5±0.1	5.0±1

Note: Values are expressed as the mean ± SE (n = 5 rats for each group). Groups received tap water (H_2O) for 60 days, aqueous extract of *Phalaris canariensis* (Pc) for 60 days, or treatment interruption after 30 days followed with tap water for another 30 days (Pc-H_2O).

Effect of indoleamine 2,3-dioxygenase inhibition

The possible role of the metabolic derivative of tryptophan, kynurenine, as a mediator of the hypotensive effect of AEPc was evaluated in adult SHR. The femoral artery was catheterized, and after 24 h, awake animals received the IDO enzyme inhibitor, 1methyl-D-tryptophan (1-MT, 50 mg·kg^{-1}, i.p.) (Wang et al. 2010). After 30 min, 400 mg·kg^{-1} of AEPc was given by gavage. Systolic blood pressure (SBP) was monitored up to 150 min as described above.

Evaluation of sympathetic vasomotor tone

For the evaluation of sympathetic neurogenic vasomotor tone, SHR or SHR treated with AEPc were submitted to acute administration of hexamethonium bromide (30 mg·kg^{-1}, i.v.) in a conscious state. The maximal fall in MAP was quantified to evaluate the contribution of sympathetic vascular tone in each group.

Experimental design: chronic treatment

Animals were assigned to 3 different groups and treated for 60 days as follows. The control groups of Wistar (W) rats, adult SHR (SHR), and young SHR (SHR_Y) received 1 mL·day^{-1} of water by gavage for 60 days. The experimental groups received AEPc (400 mg·kg^{-1}·day^{-1}, p.o.) for 30 days (W-Pc; SHR-Pc; SHR_Y-Pc), and then the animals were subdivided into a group treated with AEPc for an additional 30 days and the treatment interruption group that received distilled water instead of AEPc for another 30 days (W-Pc+H_2O; SHR-Pc+H_2O; SHR_Y-Pc+H_2O).

Blood pressure measurement in chronically treated animals

The tail SBP was measured by the tail-cuff method in conscious rats using the PowerLab system (ADInstruments). Animals were warmed up to 37 °C for 10 min, and then at least 3 determinations were made in every session of SBP measurements. The mean of the 3 values was taken as the SBP level. The BP recording was performed on the same timetable to reduce the influence of the circadian rhythm.

Table 3. Urinary volume, sodium (Na^+), potassium (K^+), and protein excretion levels in adult male Wistar (W) rats, spontaneously hypertensive rats (SHR), and prehypertensive young SHR (SHR_Y).

	Volume (mL·24 h^{-1})			Na^+ (mequiv·24 h^{-1})			K^+ (mequiv·24 h^{-1})			Protein (mg·24 h^{-1})		
Time	W	SHR	SHR_Y	W	SHR	SHR_Y	W	SHR	SHR_Y	W	SHR	SHR_Y
H_2O												
Day 0	13±1	10±1	4±0.3	2.2±0.1	1.8±0.1	0.3±0.1	4.9±0.3	4±0.4	0.5±0.1	24±2	27±3	0.4±0.2
Day 30	14±2	14±2	10±0.4a	1.7±0.3	1.4±0.2	1.9±0.1a	4.4±0.5	4±0.2	4±0.5a	23±3	34±4	16±3a
Day 60	12±1	13±1	10±0.8a	1.8±0.2	1.3±0.1	1.6±0.2a	4.9±0.3	4±0.3	4±0.3a	23±2	37±5	27±2ab
Pc												
Day 0	12±1	10±1	3±0.7	2.1±0.2	1.8±0.2	0.2±0.1	5.1±0.4	4±0.3	0.5±0.1	24±3	32±1	0.6±0.1
Day 30	15±2	10±1	10±0.7a	2.3±0.3	1.4±0.1	1.6±0.1a	5.5±0.4	4±0.2	4±0.2a	27±3	29±2	11±1a
Day 60	13±2	12±1	9.0±0.5a	1.7±0.3	1.7±0.3	1.6±0.1a	4.7±0.4	4±0.4	4±0.2a	25±1	34±3	21±2ab
Pc-H_2O												
Day 0	12±1	10±1	2±0.3	2.1±0.2	2.0±0.2	0.1±0.1	5.1±0.4	5±0.1	0.5±0.1	24±3	32±3	0.6±0.1
Day 30	15±2	09±1	11±0.8a	2.3±0.3	1.5±0.2	1.7±0.2a	5.5±0.4	4±0.3	4±0.5a	27±3	29±2	12±1a
Day 60	13±2	10±1	10±0.8a	1.8±0.3	1.6±0.1	1.7±0.2a	4.8±0.1	4±0.1	5±0.3a	26±1	34±3	21±2ab

Note: Values are expressed as the mean ± SE (n = 5 rats for each group). Groups received tap water (H_2O) for 60 days, aqueous extract of *Phalaris canariensis* (Pc) for 60 days, or treatment interruption after 30 days followed with tap water for another 30 days (Pc-H_2O). Values followed by different letters are significant at $p \leq 0.05$; a, compared with day 0; b, compared with day 30 of respective group.

Table 4. Creatinine (Cr) clearance, fractional excretion of sodium (Na^+), and fractional excretion of potassium (K^+) in adult male Wistar (W) rats, spontaneously hypertensive rats (SHR), and prehypertensive young SHR (SHR_Y).

	Cr clearance (mL·min^{-1})			Fractional excretion of Na^+ (%)			Fractional excretion of K^+ (%)		
	W	SHR	SHR_Y	W	SHR	SHR_Y	W	SHR	SHR_Y
H_2O									
Day 0	1.2±0.1	1.0±0.1	—	0.9±0.1	0.8±0.1	—	55±4	56±6	—
Day 30	1.7±0.1a	1.5±0.3	1±1	0.5±0.1a	0.5±0.1a	0.8±1	36±4a	38±5a	53±11
Day 60	1.7±0.1a	1.0±0.2	0.7±1	0.6±0.1a	0.5±0.1a	1.4±1	31±1a	44±4	58±11
Pc									
Day 0	1.4±0.1	0.9±0.1	—	0.7±0.1	0.9±0.2	—	46±4	61±8	—
Day 30	1.8±0.1	1.0±0.1	0.9±1	0.6±0.1	0.6±0.1	0.9±1	51±4	42±1	65±2
Day 60	1.6±0.2	1.0±0.1	0.9±1	0.4±0.1ab	0.7±0.1	0.95±1	37±3b	48±6	76±10
Pc-H_2O									
Day 0	1.4±0.1	0.9±0.1	—	0.7±0.1	0.8±0.1	—	46±4	60±7	—
Day 30	1.8±0.1a	1.0±0.1	0.9±1	0.6±0.1	0.6±0.1	1.0±1	51±4	46±14	66±11
Day 60	2.0±0.1a	1.0±0.1	1±1	0.4±0.1	0.6±0.1	0.8±1	27±1ab	34±2	59±4

Note: Values are expressed as the mean ± SE (n = 5 rats for each group). Groups received tap water (H_2O) for 60 days, aqueous extract of *Phalaris canariensis* (Pc) for 60 days, or treatment interruption after 30 days followed with tap water for another 30 days (Pc-H_2O). Values followed by different letters are significant at $p \leq 0.05$; a, compared with day 0; b, compared with day 30 of the respective group.

Biochemical analysis of plasma and urine

Rats were placed in metabolic cages for 24 h for urine collection before (day 0) and after 30 and 60 days of treatment. Blood samples were obtained from the retro-orbital plexus (after 30 days) and from the abdominal aorta (after 60 days). Plasma and urinary concentrations of sodium and potassium (electrolyte analyzer, Roche AVL 9180, São Paulo, Brazil), creatinine (commercial kit, Labtest Diagnostics, Lagoa Santa, Brazil) and protein (Sensiprot, Labtest Diagnostics) were determined before and after 30 or 60 days of AEPc treatment.

Statistical analysis

Results are presented as the mean ± standard error (SE). Data were evaluated by 1-way analysis of variance (ANOVA) followed by Tukey's test and the Student–Newman–Keuls test when appropriate. Results were considered statistically significant at $p \leq 0.05$.

Results

The acute effects of AEPc are shown in Fig. 1. The dose–response effect showed that the most effective dose to reduce BP was 400 mg·kg^{-1}, which induced a reduction in BP of 27 mm Hg compared with time zero. This dose was then used in the chronic experiments. The effect of AEPc on the sympathetic vasomotor tone was evaluated by analyzing the effects of acute ganglionic blockade in SHR treated or not with AEPc. The depressor response to hexamethonium administration was significantly higher in untreated SHR (76 ± 1 mm Hg decrease from a basal of 172± 6 mm Hg) compared with AEPc-treated SHR (54 ± 3 mm Hg decrease from a basal of 149± 4 mm Hg), as shown Fig. 2.

Table 1 shows the general parameters and that there were no significant differences in body mass gain or water and food intake between the untreated and treated groups. However, as

Fig. 3. (A) Effect of the aqueous extract of *Phalaris canariensis* (AEPc) on systolic blood pressure (SBP) in normotensive Wistar rats before (day 0) and after 30 and 60 days of treatment. Groups: control (W), receiving tap water; treated with AEPc for 60 days (W-Pc); and treatment interruption after 30 days and then receiving tap water for another 30 days (W-Pc+H_2O). (B) Mean variation in SBP relative to day 0 (baseline). Data are expressed as the mean ± SE ($n = 6$). $p \leq 0.05$: a, compared with day 0; b, compared with 30 days of the respective group; c, compared with the control group (W) for the same time of treatment.

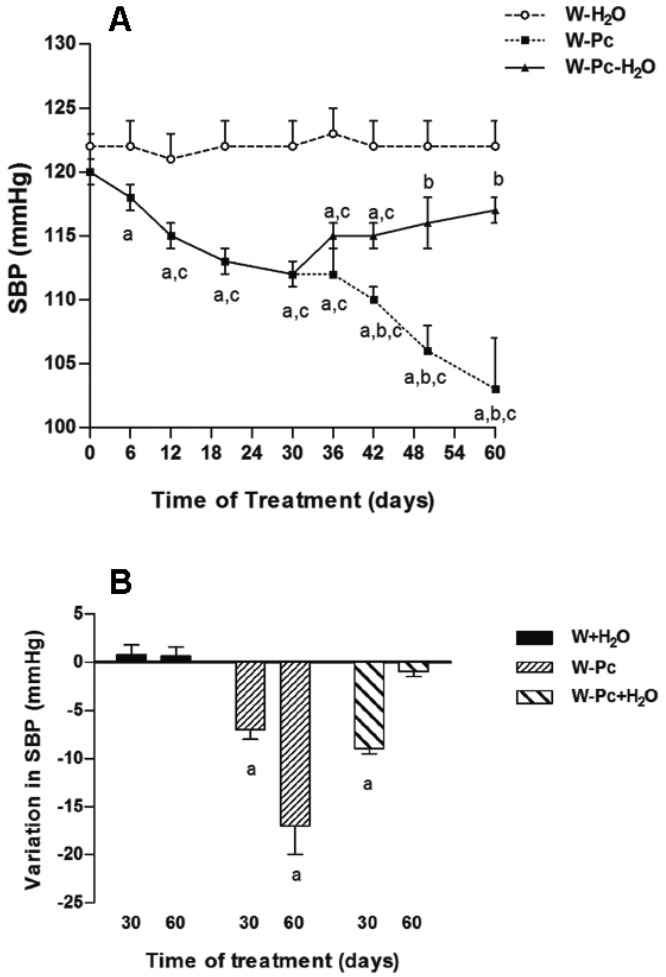
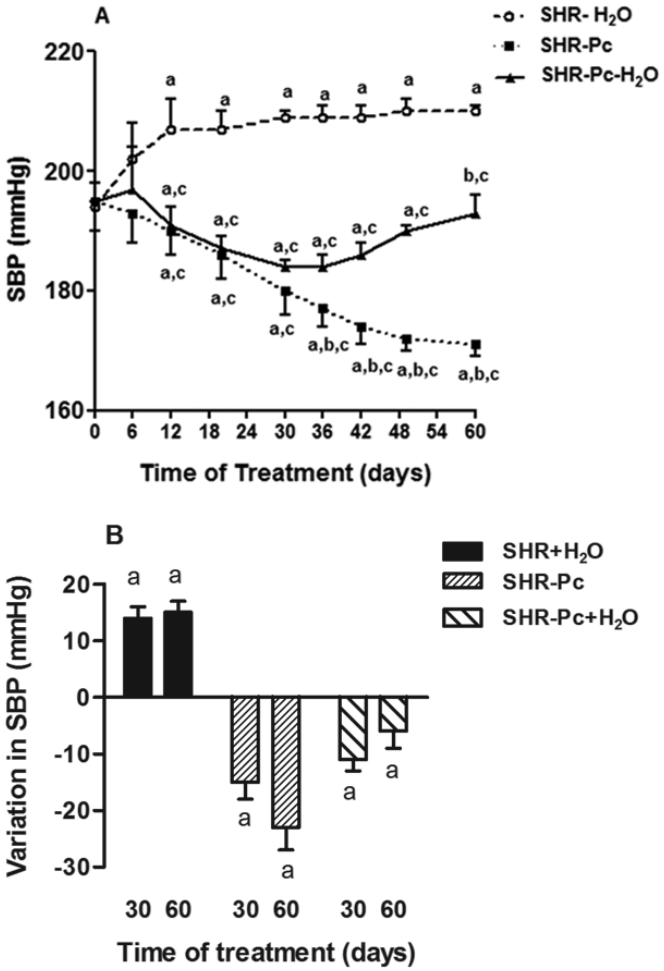


Fig. 4. (A) Effect of the aqueous extract of *Phalaris canariensis* (AEPc) on systolic blood pressure (SBP) in adult spontaneously hypertensive rats (SHR) before (day 0) and after 30 and 60 days of treatment. Groups: control (SHR), receiving tap water; treated with AEPc for 60 days (SHR-Pc); and treatment interruption after 30 days and then receiving tap water for another 30 days (SHR-Pc+H_2O). (B) Mean variation in SBP relative to day 0 (baseline). Data are expressed as the mean ± SE ($n = 5$). $p \leq 0.05$: a, compared with day 0; b, compared with 30 days of the respective group; c, compared with the control group (SHR) for the same time of treatment.



expected, there were differences among Wistar, SHR, and SHR_Y animals. Plasma parameters (Table 2), the urinary excretion of Na^+, K^+, and protein, and the 24 h urinary volume (Table 3) were also not changed by AEPc treatment. Finally, as shown in Table 4, AEPc did not change the creatinine clearance or the fractional excretion of Na^+ and K^+.

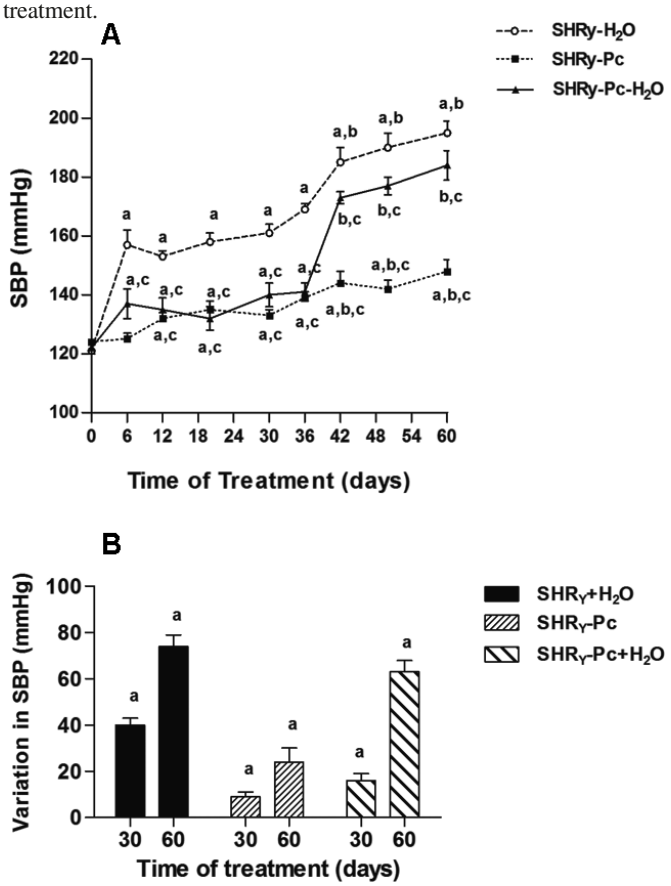
Daily administration of AEPc (400 mg·kg^{-1}·day^{-1}) induced a progressive decline in SBP after 30 and 60 days when compared with the baseline in both Wistar rats (Fig. 3A) and SHR (Fig. 4A). After 60 days, the SBP was reduced by 18% (mean ± SE: –18 ± 3 mm Hg) in the W group (Fig. 3B) and by 25% (mean ± SE: –25 ± 4 mm Hg) in the SHR group (Fig. 4B). However, the SBP increased to near baseline values with the discontinuation of treatment in both the W and SHR animals, although SBP in SHR remained lower ($p < 0.05$) at day 60 than in untreated control group.

The AEPc treatment initiated before the development of hypertension in young SHR significantly attenuated the increase in SBP compared with the untreated SHR_Y group (Fig. 5A), although the mean value obtained at day 60 was still higher ($p < 0.05$) than that for day 0. Treatment interruption allowed SBP to increase and reach levels near to those of the untreated animals ($p > 0.05$). The variation in SBP in SHR_Y is present in Fig. 5B. Control (untreated) SHR_Y animals demonstrated an increase of 60% (74 ± 5 mm Hg) in SBP, whereas, in AEPc-treated SHR_Y, the increase in SBP was significantly attenuated after 60 days (20%, 24 ± 6 mm Hg).

The amino acid profile of the AEPc analyzed by high-performance liquid chromatography confirmed that the extract is rich in tryptophan, as can be observed in Fig. 6.

The possible role of the tryptophan–kynurenine pathway in the SBP-reducing effect of AEPc was evaluated by using the IDO inhibitor 1-MT. Results shown in Fig. 7 indicate that the acute effects of AEPc were blunted by IDO inhibition.

Fig. 5. (A) Effect of the aqueous extract of *Phalaris canariensis* (AEPc) on systolic blood pressure (SBP) in young spontaneously hypertensive rats (SHR) before (day 0) and after 30 and 60 days of treatment. Groups: control (SHR_Y) received tap water; group treated with AEPc for 60 days (SHR_Y-Pc); and treatment interruption after 30 days and then receiving tap water for another 30 days (SHR_Y-Pc+H_2O). (B) Mean variation in SBP relative to day 0 (baseline). Data are expressed as the mean ± SE ($n = 5$). $p \leq 0.05$: a, compared with day 0; b, compared with 30 days of the respective group; c, compared with the control group (SHR_Y) for the same time of treatment.



Discussion

The results obtained in the present study demonstrated that the chronic administration of AEPc had no apparent systemic or renal toxic effects, nor did it interfere with the animals' growth or food and water intake.

The administration of AEPc produced a significant reduction in SBP in both adult normotensive and hypertensive rats; however, the interruption of treatment was followed by a gradual return of SBP to baseline levels at 60 days in both groups. The hypotensive effect of AEPc, observed in Wistar rats and SHR, occurred independently of any change in urinary volume or Na^+ excretion, indicating that it was not related to volume alterations. In fact, fluid and Na^+ retention do not seem to play a major role in the mechanism of hypertension in SHR; rather, it has been attributed to both neural and vascular alterations (Simpson et al. 1994). The sympathetic activity is increased in SHR, and the BP can be lowered by decreasing the sympathetic tone (de Champlain 1990; Paulis et al. 2007). Although the endothelium-dependent relaxation is preserved in young SHR (Török et al. 2006), the increased sympathetic activity in adult SHR is only partially counterbalanced by endothelium nitric oxide, which has been pointed out as the major regulatory abnormality for the genesis of hypertension in SHR (Török 2008). Taken together, these data suggest that the AEPc-induced reduction in SBP may be related to a direct vascular effect and (or) a reduction in the sympathetic vasomotor tone.

Acute experiments showed that AEPc significantly reduced sympathoexcitation in SHR. As discussed bellow, the hypotensive effect of AEPc is probably mediated by the tryptophan–kynurenine pathway. Previous studies showed that central administration of kynurenic acid decreased BP and sympathetic vasomotor tone in experimental hypertension, including the genetic model of hypertension (SHR) and angiotensin II-induced hypertension (Bergamaschi et al. 1995; Ito et al. 2000). Our hypothesis is that the depressor effect of AEPc acutely administrated in SHR is, at least in part, mediated by increased kynurenic acid release into the central nervous system, leading to sympathoinhibition. It is important to note that the inhibition of sympathetic activity observed in acute experiments may not explain the chronic effects of AEPc, whose mechanism could be distinct from that observed during acute administration, including the sympatholytic effect. Such possible mechanisms are under investigation at this moment.

It has been shown that Pc seed is an exceptional source of tryptophan (Adrian et al. 1969; Abdel-Aal et al. 1997) as we also observed in the present study. Tryptophan can be metabolized to kynurenine by either tryptophan-2,3-dioxygenase (Dick et al. 2001) or by IDO (Ball et al. 2009) depending on the tissue and cell type. It was very recently shown that the IDO-mediated endothelium-dependent metabolism of tryptophan produced arterial relaxation (Wang et al. 2010). In the same study it was shown that kynurenine administration was able to reduce BP in SHR, suggesting that the metabolism of tryptophan by IDO can constitute a pathway for the regulation of vascular tone in SHR. Based on these premises, we evaluated whether the hypotensive effect of Pc would be related to the tryptophan metabolism via IDO. In fact, the previous administration of 1-MT, an IDO inhibitor, to SHR prevented the reduction of SBP induced by AEPc, suggesting a potential role of this metabolic pathway in the AEPc-BP reducing effect. It has been shown that the tryptophan degradation to kynurenine is related to an increase in the activity of the inducible oxide nitric synthase (Kujundzić and Lowenthal 2008) and thus with NO production; however, the role of NO as a mediator of the hypotensive property of AEPc needs further investigation.

The results of the present study showed that the aqueous extract of *P. canariensis* has hypotensive effects in both normotensive and hypertensive rats. Suppression of sympathetic vascular tone is a likely mechanism involved in the acute response. The metabolism of tryptophan to kynurenine via IDO may be one of the potential mediators of this effect. No detectable toxic effects of AEPc were observed, and thus AEPc may be of pharmacological interest as an adjuvant and (or) and alternative pathway to reduce BP.

Acknowledgments

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Fig. 6. Amino acid profile of the aqueous extract of *Phalaris canariensis*.

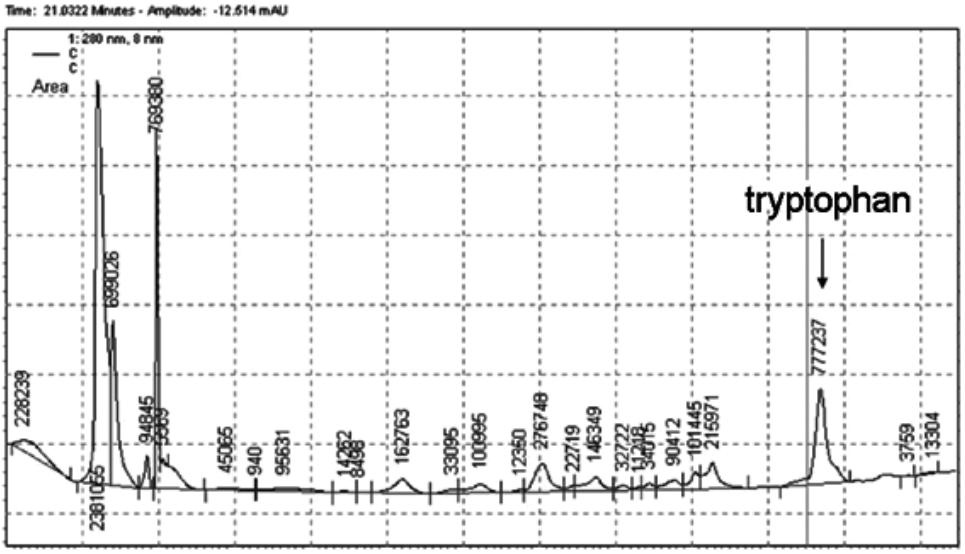
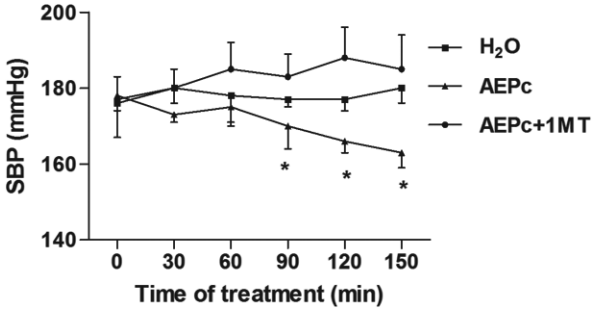


Fig. 7. Acute effect of the aqueous extract of *Phalaris canariensis* (AEPc) on systolic blood pressure (SBP) in catheterized awake spontaneously hypertensive rats (SHR) pretreated with an IDO inhibitor (1-MT). After baseline SBP measurements (time 0) animals received water or AEPc (400 mg·(kg body mass)$^{-1}$) in the absence and presence of 1-MT (50 mg·kg^{-1}); *, $p < 0.05$ compared with the control.



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