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Antihypertensive effects of isoquercitrin and extracts from *Tropaeolum majus* L.: Evidence for the inhibition of angiotensin converting enzyme

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ABSTRACT

Aim of the study: Previous studies have shown that the extracts obtained from *Tropaeolum majus* L. exhibit pronounced diuretic properties. In the present study, we assessed whether the hypotensive and/or antihypertensive mechanism of hydroethanolic extract (HETM), semi-purified fraction (TMLR) obtained from *T. majus* and the flavonoids isoquercitrin (ISQ) and kaempferol (KPF) can be mediated by their interaction with angiotensin converting enzyme (ACE).
Methods and methods: Firstly, to evaluate changes in mean arterial pressure (MAP), different groups of normotensive and spontaneously hypertensive rats (SHR) were orally and intraduodenally treated with HETM (10–300 mg/kg) and TMLR (12.5–100 mg/kg) and intravenously treated with ISQ and KPF being later anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg). The left femoral vein and the right carotid artery were isolated, and polyethylene catheters were inserted for ISQ and KPF (0.5–4 mg/kg) administration and blood pressure recording, respectively. The plasmatic ACE activity was evaluated to indirect fluorimetry, in serum samples after orally treatment with HETM, TMLR, ISQ and KPF.
Results: The oral administration of the HETM and its TMLR significantly reduced, in a dose-dependent manner, the MAP in both normotensive and SHR. In addition, these preparations significantly decreased the MAP for up to 3 h after the administration of the extract. Additionally, the intravenous administration of ISQ, but not KPF, decreased MAP in rats. Otherwise, neither the extracts nor ISQ affected the heart rate. The oral administration of the HETM, TMLR or ISQ reduced ACE activity in serum samples at 90 min after administration. Finally, the intravenous administration of ISQ caused a significant reduction in the hypertensive response to angiotensin I, but not angiotensin II in normotensive rats.
Conclusion: Our results show that the hypotensive effects caused by the HETM, as well as by its TMLR, may be associated with the high levels of the flavonoid ISQ found in this plant. In addition, ISQ-induced hypotension in rats is an event dependent on the inhibition of angiotensin II generation by ACE.

1. Introduction

Cardiovascular disease manifests in a number of clinically relevant ways, affecting a large segment of the world's adult population (Zhang et al., 2006). Patients with a blood-pressure measure of 140/90 mm Hg (systolic/diastolic pressure) are typically regarded as having high blood pressure, as defined in guidelines. Hypertension also involves complex structural and functional alterations in target organs. In the hypertensive state, a number of adaptive changes occur in blood vessels, leading to endothelial dysfunction and an increase in the extracellular matrix (Addison et al., 2008). Common clinical strategies to achieve a lowering of blood pressure include the use of beta adrenergic antagonists, calcium channel blockers, diuretics, angiotensin II type I receptor antagonists and angiotensin converting enzyme (ACE) inhibitors (Williams et al., 2004).

Inhibition of ACE has been a highly successfully program in the pharmaceutical industry, resulting in a class of effective antihyper-

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tensive drugs. In fact, synthetic ACE inhibitors such as captopril and enalapril are widely used for the treatment of cardiovascular and renal disease, for the secondary prevention of coronary artery disease, and for heart failure (Pfeffer and Frohlich, 2006). However, side effects such as cough, angioneurotic edema and deleterious effects in pregnancy have been associated with the clinical use of ACE inhibitors (Israili and Hall, 1992; Opie, 1996).

A number of extracts and compounds obtained from plants have been identified as *in vitro* ACE inhibitors (Nyman et al., 1998; Park et al., 2003). Nevertheless, only a few studies on ACE inhibitory activity underlying the hypotensive and antihypertensive effects of plant species have been reported.

Flavonoids are ubiquitously distributed throughout nature, especially in certain plants, and are often utilized in folk medicine in many countries. The finding that the consumption of certain flavonol-rich foods can mediate a reduction in blood pressure raises the possibility that flavonols may act as pharmacological ACE inhibitors. Several studies have shown that some flavonoids exhibit *in vitro* ACE inhibition (Emura et al., 2007; Lacaille et al., 2001; Schramm and German, 1998; Wu and Muir, 2008). In addition, it has been shown that some flavonoids also possess other relevant pharmacological properties, such as antioxidant and cardioprotective actions (Wu and Muir, 2008).

Tropaeolum majus L. (Tropaeolaceae), popularly known as "chaguinha", is well-recognized in traditional medicine as having cardiovascular and renal effects (Ferreira et al., 2004; Ferro, 2006; Lorenzi and Matos, 2002), although no scientific data have been published to support these effects. Previous findings from our research group have shown that extract from *Tropaeolum majus* exhibit has important *in vivo* diuretic properties (Gasparotto Junior et al., 2009). In relation to potential cardiovascular activities, experimental studies indicated potential antithrombin (De Medeiros et al., 2000) and *in vitro* ACE inhibitor activities (Han et al., 1991).

In the present study, we have further investigated the presence of isoquercitrin and kaempferol in a hydroethanolic extract (HETM) and semi-purified fraction (TMLR) from *Tropaeolum majus* and whether or not the hypotensive/antihypertensive actions of the HETM, TMLR, isoquercitrin and kaempferol are associated with their ability to inhibit ACE *in vivo*.

2. Materials and methods

2.1. Drugs

N-hippuryl-L-histidyl-L-leucine hydrate, o-phthalaldehyde, angiotensin I, bradykinin, isoquercitrin and kaempferol were obtained from Sigma (St. Louis, MO, USA). Captopril from Sigma (St. Louis, MO, USA) was used as the reference ACE inhibitor drug. Other drugs and reagents used were of analytical grade.

2.2. Phytochemical studies

2.2.1. Plant material and preparation of the HETM and TMLR

Tropaeolum majus leaves were collected in June 2008 from the botanical garden of Universidade Paranaense (UNIPAR), Umuarama (Brazil) at 430 m above sea level (S23°47′55–W53°18′48). The plant was identified by Dr. Mariza Barion Romagnolo (Department of Botany, Universidade Paranaense, PR, Brazil). Voucher specimens were deposited at the Herbarium of this University under number 2230. Crude ethanolic extract (HETM) and fraction (TLMR) were obtained as previously described (Gasparotto Junior et al., in press). Briefly, the extraction with ethanol 90% yielded HETM, which was submitted to a column with Amberlite XAD-2 to give TLMR. Both samples were dissolved in distilled water immediately prior to administration.

2.2.2. Phytochemical analysis

HETM and TLMR were analyzed by HPLC and ESI-MS using the same equipments previously reported (Gasparotto Junior et al., in press). HETM and TMLR were dissolved in a solution containing 50% (v/v) chromatographic grade methanol and 50% (v/v) deionized water with 0.5% ammonium hydroxide (Merck, Darmstadt, Germany). In the fingerprinting ESI-MS analysis, the general conditions were as follows: source temperature of 100 °C; capillary voltage of 3.0 kV; and cone voltage of 30 V. For measurements in the negative ion mode, ESI(−)-MS, 10 μL of concentrated NH_4OH were added to the sample mixture (having a total volume of 1000 μL), yielding 0.1% as final concentration. ESI-MS was preformed by direct infusion with a flow rate of 10 μL min mL^{-1} using a syringe pump (Harvard Apparatus). Structural analysis of single ions in the mass spectra from extract and fraction was performed by ESI-MS/MS. The ion with the m/z of interest was selected and submitted to 15–45 eV collisions with argon in the collision quadrupole. The collision gas pressure was optimized to produce extensive fragmentation of the ion under investigation. The compounds were identified by comparison of their ESI-MS/MS fragmentation spectra with literature data (Roesler et al., 2008; Ye et al., 2007). In addition the chemical composition of HETM and the TMLR were also determined by HPLC-UV using comparative analysis using isolated standards. A volume of 10 μL of each sample was injected into the RP-18 (Lichrospher®, Merck KGaA, Darmstadt, Germany) column (5 μm, 225 mm × 4.6 mm i.d.) protected by a RP-18 guard column from Merck (5 μm, 4.0 mm × 3.0 mm i.d.). The mobile phase consisted of a linear gradient combining solvent A (acetonitrile) and solvent B (water/acetic acid, 99:1, v/v, pH 2.88) as follows: 15% A (15 min), 15–20% A (7 min), 20% A (5 min), 20–40% A (5 min), 40% A (5 min), 40–15% A (5 min). The analyses were carried out in triplicate, at a flow rate of 0.6 mL/min and peak areas were calculated by a Shimadzu CLASS-VP™ 7.2.1. For the bioactive compound isoquercitrin, HPLC quantitative analysis was performed (Li et al., 2008).

2.2.3. Quantitative HPLC analysis of isoquercitrin

In order to prepare standard solutions, isoquercitrin standard was dissolved in methanol/water (1:1, v/v). Serial concentrations of this flavonoid were prepared at eight concentrations, from 1 to 20 μg/mL. Quantitative HPLC analyses were conducted using the same conditions described above. The analyses were carried out in triplicate, at a flow rate of 0.6 mL/min with the UV detector set at λ 330 nm and an injection volume of 10 μL. Calibration graphs were plotted showing a linear relationship between concentrations versus peak areas for the reference compound.

Accurately weighed amounts of the HETM and TMLR were dissolved in methanol/water (1:1) and analyzed using the same chromatographic conditions as used for isoquercitrin. The attribution of the chromatographic peak was based on the retention times of the single compound and confirmed by analysis in comparison with the isolated standard. Under our working conditions, the mean of retention time for isoquercitrin was 33.75 min. The concentration of the isoquercitrin was calculated from the experimental peak area by analytical interpolation on a standard calibration line. The limit of quantification (LOQ) for the compound analyzed was the 1.25 μg/mL.

2.3. Pharmacological studies

2.3.1. Animals

We used male Wistar rats (3–4 months old, weighing between 250 and 300 g) from the colony of the Universidade Federal do Paraná. Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) were obtained from Biomedical Science Institute of the Universidade de São Paulo (USP), Brazil. All animals were main-

tained under standard laboratory conditions, with a constant 12 h light/dark cycle and controlled temperatures (22 ± 2 °C). Standard pellet food (Nuvital®, Curitiba/PR, Brazil) and water were available *ad libitum*. All experimental procedures adopted in this study were previously approved by the Institutional Ethics Committee of the Universidade Federal do Paraná (authorization number 240).

2.3.2. Direct blood pressure and heart rate measurement in anesthetized rats

Normotensive male Wistar rats, WKY or SHR were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg), administered intramuscularly and supplemented at 45–60 min intervals. A polyethylene catheter was inserted into the right femoral vein for flavonoids and drug administration. Immediately after venous cannulation, a bolus injection of heparin (30 IU) was administered i.v. Animals were allowed to breath spontaneously through a tracheotomy. The left carotid artery was cannulated and connected to a pressure transducer coupled to a PowerLab® recording system, and an application program (Chart, v 4.1; all from ADI Instruments; Castle Hill, Australia) recorded both mean arterial pressure (MAP) and heart rate (HR). For stabilization of the blood pressure after the surgical process, an interval of 15 min was held before the injection of any drug. At the end of the experiments, animals were killed with an overdose of thiopental (over 40 mg/kg, i.v.).

2.3.3. Experimental protocols

2.3.3.1. Hypotensive dose response and time course of HETM and TMLR obtained from Tropaeolum majus. Different groups of normotensive rats received orally (10–300 mg/kg), TMLR (12.5–100 mg/kg) or the vehicle via an oral route, at a constant volume of 100 μL/100 g body weight. MAP and HR values for each animal were recorded after 1.5, 3, 6 and 12 h of treatment. Each animal received only one of the substances studied.

2.3.3.2. Evaluation of antihypertensive effects of HETM and TMLR obtained from Tropaeolum majus. Groups of WKY and SHR, prepared for direct blood pressure measurements as previously described, received intraduodenally HETM or TMLR at 100 and 50 mg/kg, respectively. The control group received the vehicle intraduodenally at a constant volume of 100 μL/100 g of body weight. The changes in MAP and HR were recorded for 45 min after these treatments.

2.3.3.3. Evaluation of hypotensive effects of isoquercitrin and kaempferol. Different groups of animals received intravenous injections of isoquercitrin (ISQ) or kaempferol (KPF), at 0.5, 1, 2 and 4 mg/kg. Administration of these flavonoids was done randomly, at a constant volume of 100 μL/100 g (body weight). Each animal was tested for only one of the flavonoids studied.

2.3.3.4. Angiotensin converting enzyme (ACE) assay. ACE activity was determined in serum plasma obtained from normotensive, male Wistar rats (250–300 g) 1.5 h after oral treatment with captopril (20 mg/kg), HETM (30–300 mg/kg), TMLR (25–100 mg/kg), isoquercitrin and kaempferol (5–10 mg/kg), as previously described (Santos et al., 1985). The control group received vehicle at a constant volume of 100 μL/100 g of body weight. Blood was collected into glass tubes after decapitation, and serum was separated after 2 h at 37 °C. Briefly, serum (10 μL) was incubated with 490 μL of assay solution containing 5 mM Hip-His-Leu in 0.4 M sodium borate buffer, pH 8.3, and 0.9 M NaCl for 15 min at 37 °C. The reaction was stopped by addition of 1.2 mL of NaOH 0.34 N. The product, His-Leu, was measured fluorometrically (365 nm excitation and 495 emission, Aminco Model J4-7461 fluoromonitor, American Instrument Co., Silver Springs, MD) after the addition of 100 μL of o-phthaldialdehyde (20 mg/ml) in methanol, which was followed 10 min later by the addition of 200 μL of HCl 3 N and centrifugation at 800 × *g* for 5 min at room temperature. To correct the intrinsic fluorescence of the plasma, time-zero blank samples were prepared by adding plasma after NaOH treatment. Captopril was used as a positive control. All measurements were made in triplicate.

2.3.3.5. Evaluation of isoquercitrin influence in angiotensin I and bradykinin-induced changes in MAP. After cannulation and MAP stabilization, the effects of angiotensin I (10 pmol/kg, i.v.), bradykinin (30 nmol/kg, i.v.) and isoquercitrin (4 mg/kg, i.v.) on the mean arterial pressure were recorded. After the administration of isoquercitrin, the injection of angiotensin I and bradykinin was repeated for comparison. An interval of 15 min between each administration was maintained.

2.4. Statistical analysis

Data for quantitative HPLC analysis are reported as means (percent of relative standard deviation) of triplicate determinations. The changes in the MAP are expressed as mean ± standard error of the mean of six experiments. Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Bonferroni's test or Student's *t*-test, when applicable. A *p*-value less than 0.05 was considered to be statistically significant. The graphs were drawn, and the statistical analyses were performed using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA).

3. Results

3.1. Phytochemical analysis

The ESI-MS fingerprints of the HETM and TMLR in the negative mode (Fig. 1) showed characteristic distributions of the flavonoids isoquercitrin (*m*/*z* 464) and kaempferol glucoside (*m*/*z* 448), which were detected as the deprotonated molecules of *m*/*z* 463 and 447, respectively, and identified by comparison with data found in the literature (Roesler et al., 2008). These results were confirmed by HPLC-UV analysis using isolated standards, and the structures were unequivocally confirmed by co-injection of authentic standards and identified by retention values (Fig. 2).

The HPLC quantitative method was validated for isoquercitrin (Gaskell, 1997), and the content of this compound in the HETM and in the TMLR was 3.86% and 9.35%, respectively (Table 1). This chemical results for *Tropaeolum majus* was recently reported (Gasparotto Junior et al., in press).

3.2. Hypotensive effects of HETM and TMLR in normotensive rats

The basal MAP recorded in anesthetized control rats, after the 15 min period allowed for stabilization, was 113.5 ± 2.4 mm Hg. The oral administration of HETM (10 and 300 mg/kg), after 1.5 h of treatment caused a significant reduction (~13 mm Hg) in MAP, in a dose and time-dependent manner (Fig. 3A and B). A significant decrease in the mean arterial pressure was observed up to 3 h after the administration of the extract (Fig. 3B). Similarly, the oral administration of TMLR (12.5 and 100 mg/kg) was able to induce dose-dependent hypotensive effects (Fig. 3C), with values of reduction of 17.94 ± 4.70 and 20.77 ± 5.43 mm Hg, respectively. At least in the range of doses used in our experiments, neither HETM nor TMLE were able to significantly reduce the heart rate of anesthetized normotensive rats (Table 2).

3.3. Antihypertensive effect of HETM and TMLR in SHR rats

The basal MAP recorded in SHR- and WKY-anesthetized rats after the stabilization period and before the administration of

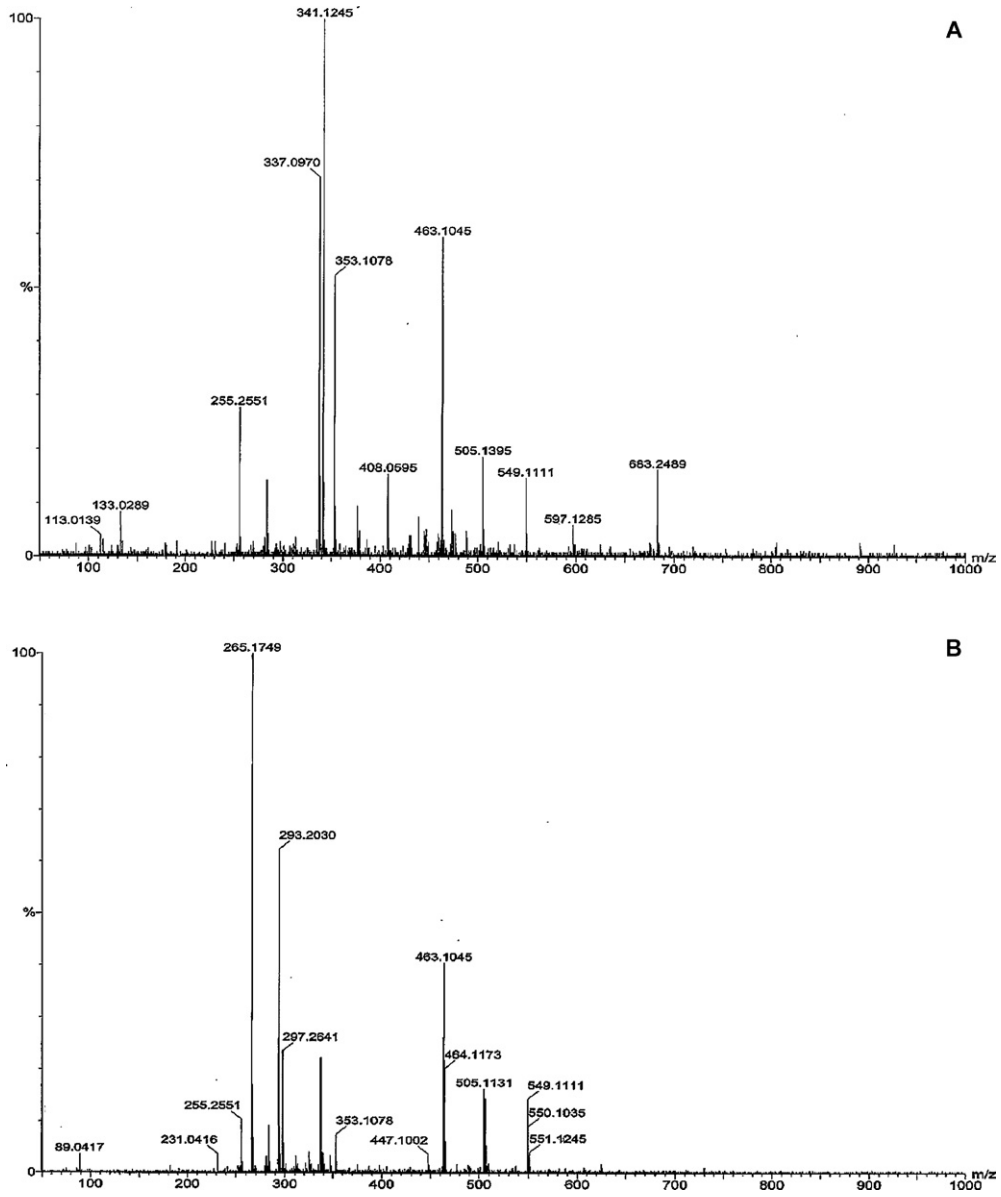

Fig. 1. ESI(−)-MS fingerprints of HETM (A) and TMLR (B).

any drug was 134.4 ± 1.8 mm Hg and 108.9 ± 1.3 mm Hg, respectively. A pronounced antihypertensive and hypotensive effect of intraduodenal administration of TMLR (50 mg/kg) or HETM (100 mg/kg) was observed in both SHR and WKY (Fig. 4A and B), with MAP reduction in the hypertensive rats of 18.77 ± 4.43 and 14.14 ± 6.7 mm Hg, respectively. Similar to the absence of influence seen in normotensive Wistar rats, HETM and TMLR did not change the heart rate in SHR or WKY animals (Table 2).

3.4. Hypotensive effects of Isoquercitrin and kaempferol in normotensive rats

The intravenous administration of isoquercitrin (0.5–4 mg/kg), but not kaempferol, caused a dose-dependent reduction in MAP (Fig. 5A and B), with minor (not statistically significant) effects on heart rate (Table 2). Doses at 2 and 4 mg/kg of isoquercitrin were able to induce significant hypotension (Fig. 5B), with values of 16.4 ± 4.3 and 23.2 ± 3.7 mm Hg, respectively.

Table 1
Isoquercitrin content of HETM and TMLR from *Tropaeolum majus*.

Sample	Isoquercitrin content[a]
HETM	38.64 (0.30)
TMLR	93.45 (2.80)

[a] Isoquercitrin content (mean of triplicate analysis), expressed as mg/g of dried extract or fraction (% RSD, relative standard deviation).

3.5. Measurement of serum ACE activity

The oral administration of HETM (0–300 mg/kg), semi-purified fraction (25–100 mg/kg) or isoquercitrin (5–10 mg/kg) significantly reduced the serum activity of ACE at 1.5 h after administration, when compared to control group (Fig. 6A–C). There was no significant difference in ACE activity in the group treated with kaempferol when compared to control animals (Fig. 6D). The percentage of ACE inhibition activity in HETM-treated groups was 24 ± 5% and 20 ± 4% at 300 and 100 mg/kg, respectively (Fig. 6A). In normotensive rats

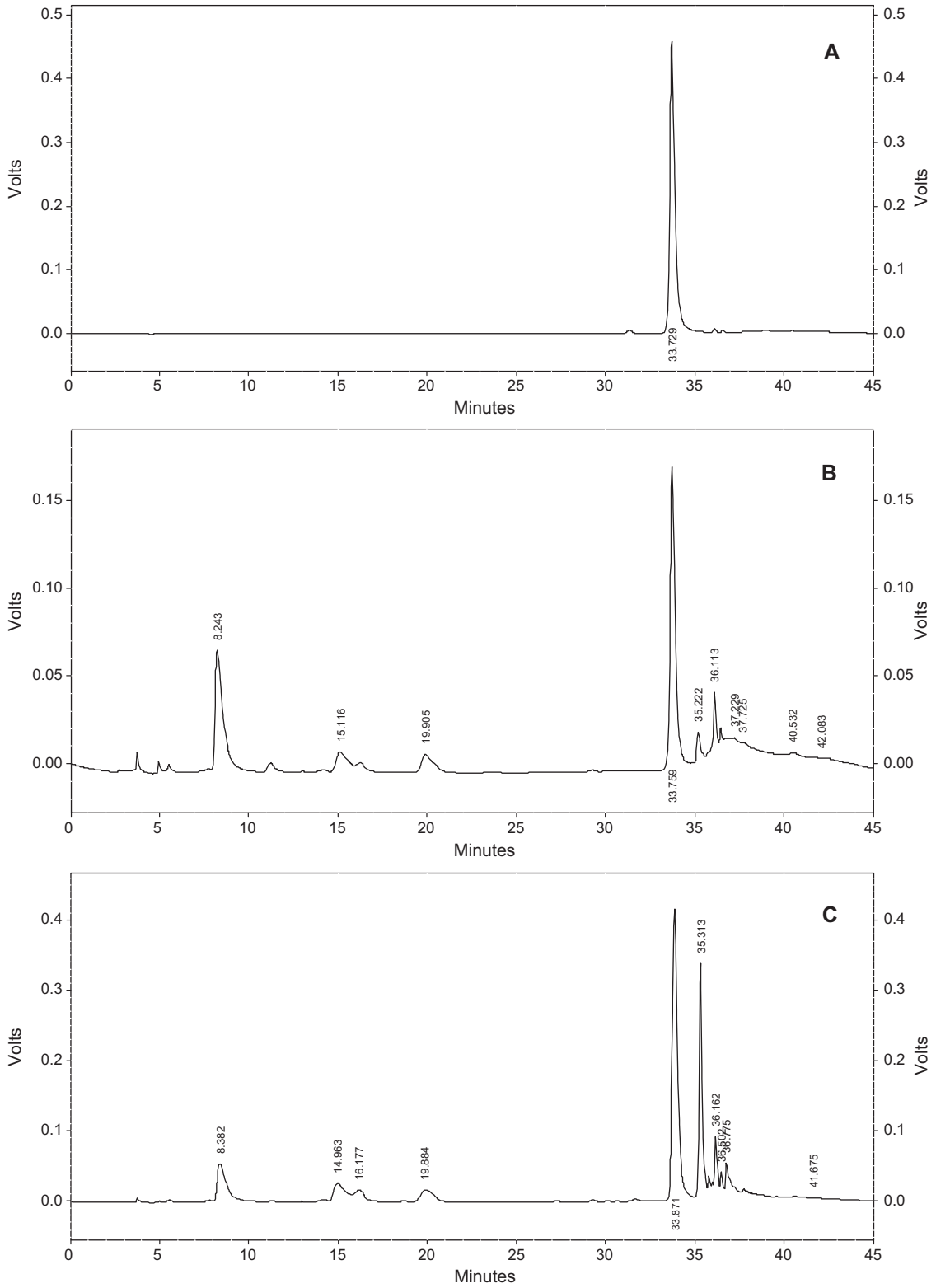

Fig. 2. HPLC chromatograms of isoquercitrin (A), HETM (B) and TMLR (C).

treated with TMLR at 100 and 50 mg/kg, ACE activity was reduced by 30 ± 8% and 28 ± 7%, respectively (Fig. 6B). Similar to captopril (reduced at 45 ± 5%), a reference ACE inhibitor, a single dose of isoquercitrin 5 and 10 mg/kg decreases ACE activity, when compared with to the control at 1.5 h after treatment (Fig. 6C). The percentage decrease of ACE activity in the isoquercitrin-treated group was 44 ± 4% and 22 ± 8% at doses 10 and 5 mg/kg, respectively.

3.6. Effects of isoquercitrin administration in MAP changes, and the time course response of angiotensin I and bradykinin

The intravenous administration of isoquercitrin (4 mg/kg) caused a significant reduction (34 ± 3%) in the hypertensive response of angiotensin I in normotensive rats (Fig. 7A). Similarly, the time course of the hypertensive response was significantly

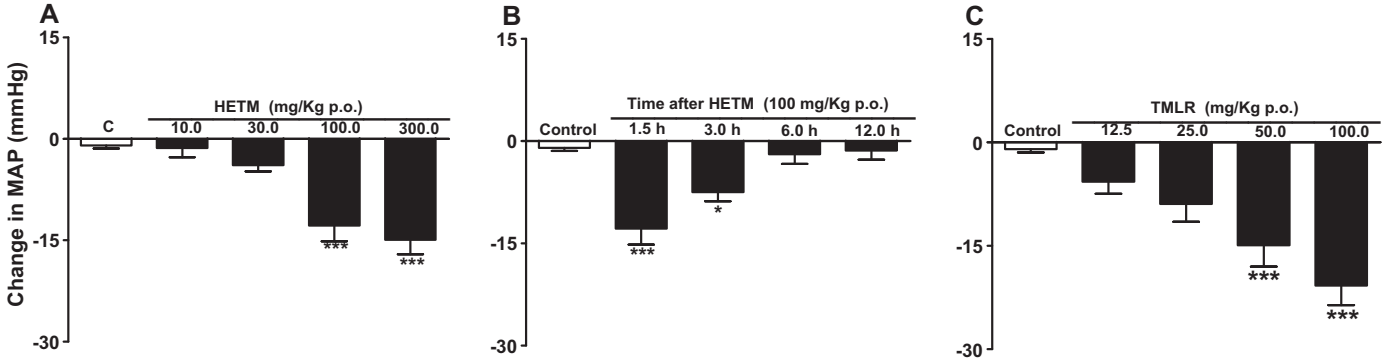

Fig. 3. Time and dose-dependent hypotension induced by HETM (A and B) and TMLR (C) obtained from *Tropaeolum majus*. The HETM or the TMLR were administered v.o. in anesthetized, normotensive rats. The "control" indicates the effect measured after administration of vehicle (distilled water) only. The results show the mean ± S.E.M. ($n = 6$). Statistical analyses were performed by means of one-way analysis of variance (ANOVA) followed by *t*-tests subjected to Bonferroni's correction. $^{*}p < 0.05$ and $^{***}p < 0.001$ when compared to control group.

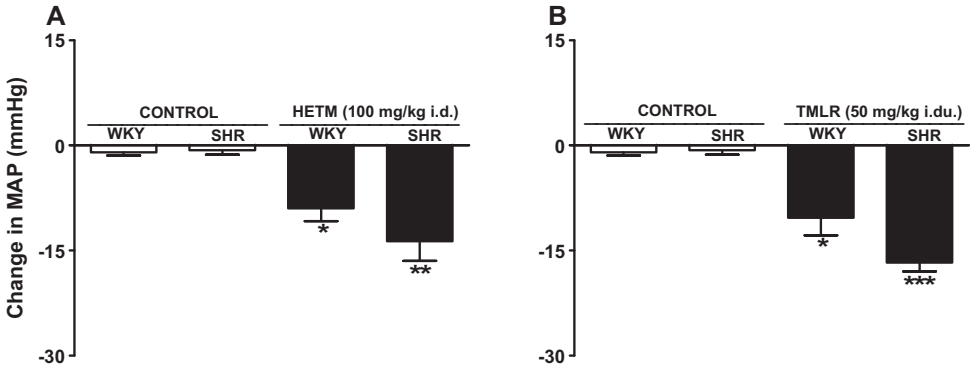

Fig. 4. Antihypertensive and hypotensive effects of HETM (A) and TMLR (B) obtained from *Tropaeolum majus* in SHR and WKY rats. The animals were subjected to an intraduodenal treatment with HETM (A) or TMLR (B). The antihypertensive and hypotensive effects were measured in these animals after 30 min of administration of distilled water (open bars) or extracts (closed bars). The results are the mean ± S.E.M. of six experiments in each group. Statistical analyses were performed by Student's *t*-test. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ when compared with the respective control group.

Table 2
Heart rate (beats per minute) of anesthetized rats treated with HETM, TMLR and isoquercitrin.

Treatment	Normotensive	SHR
Control	271 ± 19 (6)	340 ± 16 (6)
HETM (100 mg/kg)	257 ± 22 (6)	310 ± 14 (6)
HETM (300 mg/kg)	246 ± 14 (6)	n.e.
TMLR (50 mg/kg)	257 ± 19 (6)	313 ± 18 (6)
TMLR (100 mg/kg)	238 ± 25 (6)	n.e.
Isoquercitrin (2 mg/kg)	265 ± 21 (6)	n.e.
Isoquercitrin (4 mg/kg)	233 ± 17 (6)	n.e.

The values are the mean ± S.E.M. of heart rate after administration of HETM, TMLR or isoquercitrin. The number of experiments per group is showed in parentheses. "n.e." indicates that the specific dose was not evaluated in the respective group.

reduced in comparison with the control group (Fig. 7B) (2.28 ± 0.17 and 3.12 ± 0.17 min, respectively). Doses at 4 mg/kg of isoquercitrin did not cause any significant change in the hypotensive effects of bradykinin (30 nmol/kg) (Fig. 7A). However, the time course of the hypotensive response was significantly increased when compared to the control group (Fig. 7B) (4.91 ± 0.27 and 3.10 ± 0.26 min, respectively).

4. Discussion

The fresh leaves and preparations obtained from *Tropaeolum majus* have been used for both nutritional and medicinal purposes in Brazil. In Brazilian folk medicine it is commonly indicated against cardiovascular disorders, such as hypertension, without description of side effects (Ferreira et al., 2004; Ferro, 2006; Lorenzi and Matos, 2002). However, the efficacy of *Tropaeolum majus* as an antihypertensive agent has never been investigated. We have recently showed that the hydroethanolic extract obtained from *Tropaeolum majus* leaves, which contains high levels of flavonoids, possesses natriuretic and diuretic effects when orally administered in normotensive rats, without acute toxicity or interference in renal functions (Gasparotto Junior et al., 2009). Based on these findings, we investigated the effects of preparations obtained from *Tropaeolum majus* in the arterial pressure of both normotensive and hypertensive (SHR) animals. Our results revealed that the hydroethanolic extract of *Tropaeolum majus* (HETM), as well as its semi-purified fraction (TMLR), are able to dose-dependently reduce the mean arterial pressure of normotensive and SHR animals (maximal hypotensive effect ~15–20 mm Hg) when administered by intraduodenal route. Importantly, at least based in our experiments, the reduction in blood pressure cannot be directly attributed to any cardiac effect of these preparations, since the hypotension measured after HETM or TMLR administration have not been accompanied by any significant reduction in the heart rate of the animals tested.

Previous phytochemical studies showed that the major metabolites in the leaves of *Tropaeolum majus* are glucosinolates and polyphenols, including flavonoids and terpenoids (De Medeiros et al., 2000; Griffiths et al., 2001; Kjaer et al., 1978; Lykkesfeldt and Moller, 1993; Mietkiewska et al., 2004). Using HPLC and ESI-MS techniques we verified that both HETM and TMLR present high levels of flavonols isoquercitrin and kaempferol glycoside (Gasparotto Junior et al., in press). Considering the lack of biolog-

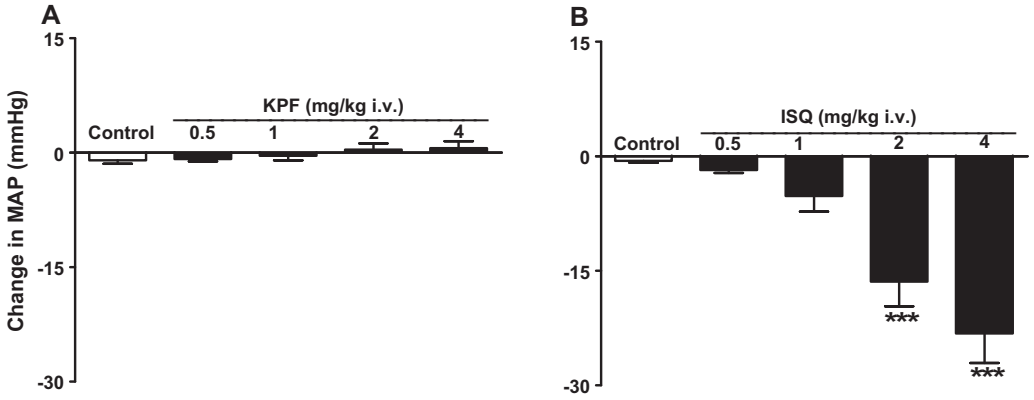

Fig. 5. Dose-dependent hypotension induced by kaempferol (KPF) and isoquercitrin (ISQ) in normotensive rats. The KPF (A) or ISQ (B) were administered i.v. in normotensive rats. The "control" indicates the effect measured after intravenous administration of vehicle (saline 0.9%) only. The results show the mean ± S.E.M. ($n = 6$). Statistical analyses were performed by means of one-way analysis of variance (ANOVA), followed by t-tests subjected to Bonferroni's correction. $^{***}p < 0.001$ when compared to control group.

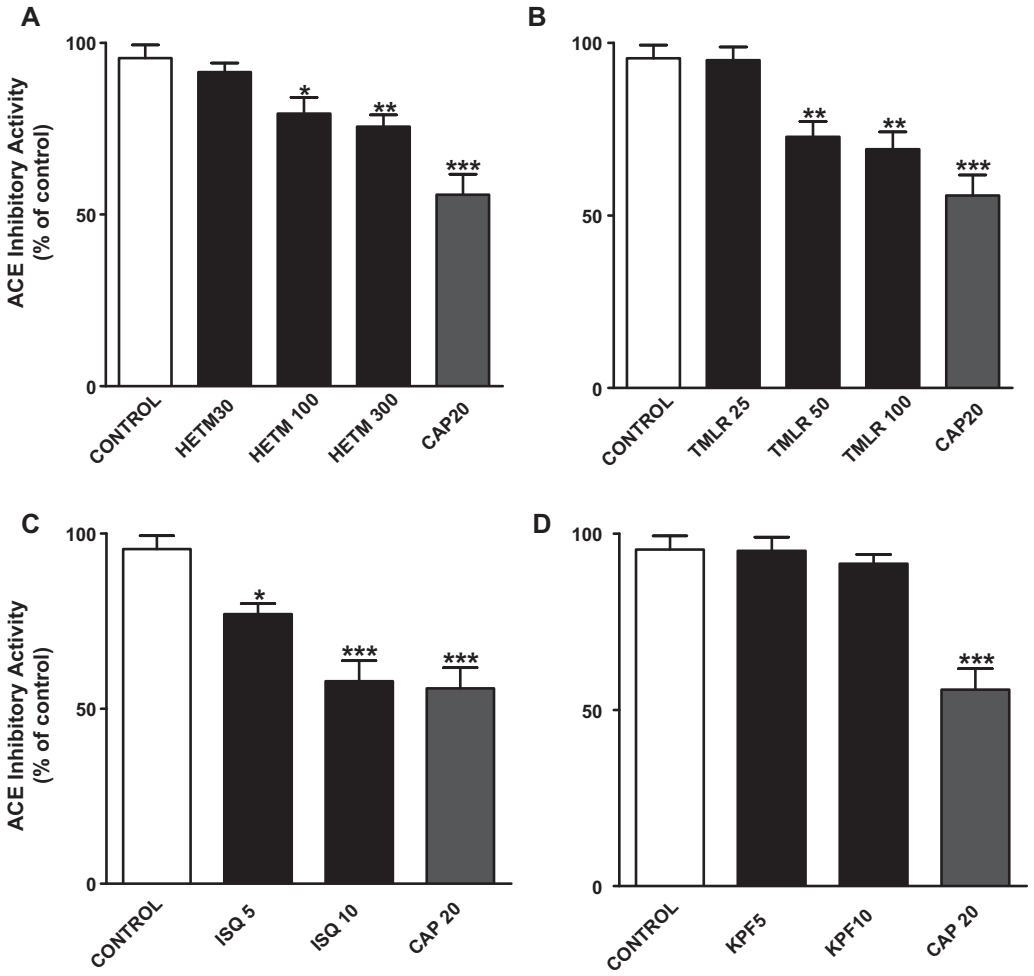

Fig. 6. Dose-dependent inhibition of the HETM (A), TMLR (B), ISQ (C) and KPF (D) on ACE serum activity. All substances tested were administered v.o. in conscious rats. The reference drug indicates the effect measured after administration of captopril (CAP, 20 mg/kg). The results are the mean ± S.E.M. of six experiments in each group. Statistical analyses were performed by means of one-way analysis of variance (ANOVA), followed by t-tests subjected to Bonferroni's correction. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ when compared to control group.

ical activities attributed to glucosinolates (Wielanek and Urbanek, 2006), we hypothesized that these polyphenolic compounds could be responsible for the hypotensive effects elicited by HETM and TMLR. In fact, the intravenous administration of low doses of isoquercitrin (but not kaempferol) administration was able to cause a significant drop in the mean arterial pressure of anesthetized rats (~25 mm Hg), remembering the effect of HETM and TMLR in blood pressure. Several flavonoids have been shown as cardioprotective and hypotensive agents in animal models (Jadhav et al., 2010; Pechanova et al., 2006; Wright et al., 2007; Yung et al., 2008). Isoquercitrin or plants containing high amounts of this flavonol are described as able to stabilize atherosclerotic plaques (Enkhmaa

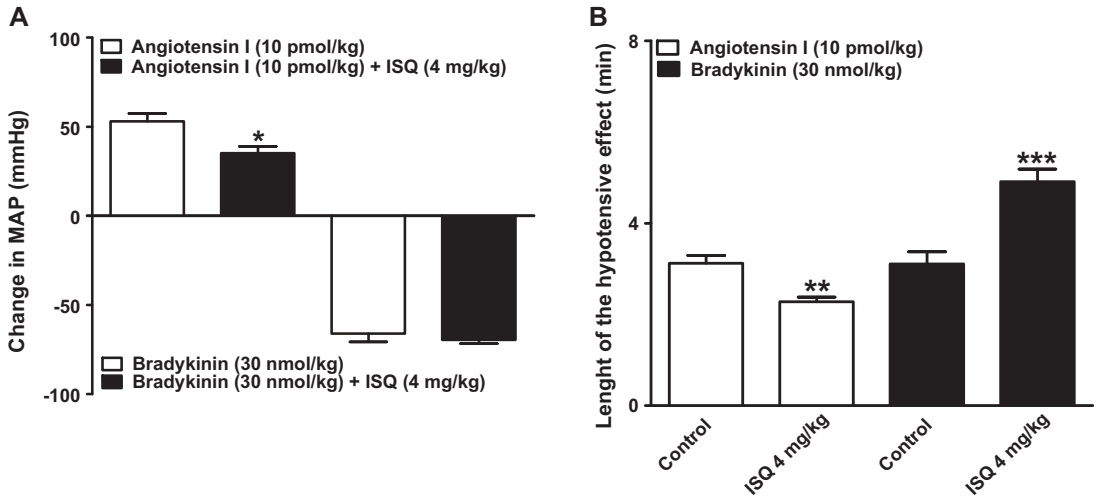

Fig. 7. Effects of isoquercitrin administration in MAP changes (A) and time-course response (B) of angiotensin I and bradykinin. All substances tested were administered i.v. in anesthetized rats. The control group indicates the effect measured before administration of ISQ (4 mg/kg). The results show the mean ± S.E.M. ($n = 6$). Statistical analyses were performed by *t*-tests. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ when compared to control group.

et al., 2005; Motoyama et al., 2009). In addition, different studies have evidenced antioxidant effects of isoquercitrin (Kim et al., 2007; Silva et al., 2009; Vitor et al., 2004). Although these actions may be related to protective cardiovascular events, this is the first study demonstrating that isoquercitrin, acutely administered, is also able to reduce blood pressure. The polyphenolic compound isoquercitrin is a flavonol glycoside (quercetin-3-O-glycoside) found in abundance in several edible and medicinal plants. It is relatively soluble and has an high absorption rate by the gastrointestinal tract (Chang et al., 2005). Since isoquercitrin was found as the main compound in the semi-purified fraction of HETM, we suggest that the anti-hypertensive effects popularly attributed to *Tropaeolum majus* may be related, at least in part, to the presence of isoquercitrin in this plant.

A few reports have suggested that aqueous solutions obtained from aerial parts of *Tropaeolum majus* present a weak *in vitro* ACE inhibitory activity (Han et al., 1991). Likewise, isoquercitrin was described as a possible inhibitor of ACE activity *in vitro* (Lacaille et al., 2001). ACE is a decapeptidyl peptidase (protease) that is widely distributed in the cardiovascular system and in various non-cardiovascular tissues. One of the main locations of the enzyme is the serum and the vasculature, where activity of ACE promotes cellular proliferation (Li et al., 1997). In addition, local ACE in the kidney causes angiotensin II production, which contributes to reducing the urinary excretion of water and sodium, thereby playing an important role in the long-term stabilization of hypertension (Khosla et al., 2009). ACE is a zinc-containing peptidyl dipeptide hydrolase (Strittmatter and Snyder, 1986). The active site of ACE is known to consist of three parts: a carboxylate-binding functionality, such as the guanidinium group of arginine; a pocket that accommodates a hydrophobic side chain of C-terminal amino acid residues; and a zinc ion. The zinc ion coordinates to the carbonyl of the penultimate peptide bond of the substrate, whereby the carbonyl group becomes polarized and is subjected to a nucleophilic attack. Therefore, some flavonoids (Wagner and Elbl, 1992) were suggested to show an *in vitro* activity via the generation of chelate complexes within the active centre of ACE. Free hydroxyl groups of phenolic compounds are also suggested to be important structural moieties to chelate the zinc ions, thus inactivating the ACE activity (Chen et al., 1992). Given that the flavonols contain aromatic hydroxyl groups, it is possible that these functional groups may demonstrate an ACE inhibitory activity due to the generation of chelate complexes with zinc ions within the active center of ACE. In fact, several flavonoids have proven to competitively inhibit ACE activity (Barbosa-Filho et al., 2006; Kwon et al., 2010; Somanadhan et al., 1999).

Our study reveals that isoquercitrin may be included in the group of flavonoids able to inhibit the production of angiotensin II by ACE seen *ex vivo*, as found using plasma samples obtained from isoquercitrin treated rats. In addition, inhibition of ACE was also demonstrated *in vivo*, since a single administration of isoquercitrin reduced the hypertension induced by angiotensin I (that results from its conversion to angiotensin II by ACE), and enhanced the hypotensive action of bradykinin, a B2 receptor agonist that is physiologically degradated through the activity of the angiotensin converting enzyme. It is well known that activation of bradykinin B2 receptors may release endothelial agents such as nitric oxide and prostacyclin, contributing to the blood pressure control and cardiovascular benefits associated with currently used ACE inhibitors. In addition, the increased half-life of endogenous bradykinin promoted by ramiprilat may be responsible for the natriuretic effect generated by this ACE inhibitor (Sakamoto et al., 1994). Importantly, angiotensin II stimulates the release of aldosterone by supra-renal gland, regulating the action of this hormone in the control of body and plasmatic volumes. Thus, inhibition of ACE also reduces the reabsorption of sodium and water by kidneys, increasing diuresis and reducing the blood volume. This diuretic-like effect of ACE inhibitors is putatively responsible for at least part of the anti-hypertensive effects of these drugs. As aforementioned, it has been previously demonstrated that the hydroalcoholic extract from *Tropaeolum majus* has diuretic properties when acutely or daily administered in rats. Since isoquercitrin is the major flavonoid in *Tropaeolum majus*, it is likely that the hypotensive effect of isoquercitrin and also from HETM might be related, at least in part, with a diuretic action of this flavonol. To check this hypothesis experiments to evaluate the diuretic properties of isoquercitrin, as well as the importance of ACE inhibition in this effect, are being carried out in our laboratory.

Although our results strongly suggest that the antihypertensive effect of *Tropaeolum majus* may be, at least in part, due to an inhibitory effect of ACE by isoquercitrin, the single administration of HETM, or its semi-purified fraction TMLR, as well as isoquercitrin, did not result in any significant reduction of heart rate, an hallmark effect of ACE inhibitors (Pierdomenico et al., 2002). Under the light of our results we are unable to explain why heart rate remained

unchanged after ACE inhibition by isoquercitrin. However, it is well known that the full cardiovascular effects of ACE inhibitors may take several months to show up and the results found in our study were obtained after a single administration of isoquercitrin. On the other hand, both HETM and TMLR had their potency as hypotensive agents much more evident in SHR than in WKY rats. Hypertension is frequently associated with an increased sympathetic regulation of the vascular tone, besides neurohumoral and renal abnormalities (Sarikonda et al., 2009). Angiotensin II acts stimulating the sympathetic control of the vascular function and ACE inhibitors have a major impact controlling these changes. Taken together, these data suggest that inhibition of ACE by isoquercitrin may be taking place also in the central nervous system.

5. Conclusion

Our results show that the hypotensive effects caused by the hydroethanolic extract of *Tropaeolum majus* (HETM), as well as its semi-purified fraction (TMLR), may be associated with the high levels of the flavonoid isoquercitrin found in this plant. In addition, isoquercitrin-induced hypotension in rats is an event dependent on the inhibition of angiotensin II generation by ACE. Given that common clinical strategies to achieve a lowering of blood pressure include the use of diuretics and inhibitors of angiotensin-converting enzyme in monotherapy or in association (Gallagher et al., 2006), our findings have highlighted the therapeutic potential of *Tropaeolum majus* as a phytomedicine and the importance of substances present in this plant for the treatment of cardiovascular diseases.

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References

Addison, S., Stas, S., Hayden, M.R., Sowers, J.R., 2008. Insulin resistance and blood pressure. Current Hypertension Reports 10, 319–325.

Barbosa-Filho, J.M., Martins, V.K.M., Rabelo, L.A., Moura, M.D., Silva, M.S., Cunha, E.V.L., Souza, M.F.V., Almeida, R.N., Medeiros, I.A., 2006. Natural products inhibitors of the angiotensin converting enzyme (ACE): a review between 1980–2000. Revista Brasileira de Farmacognosia 16, 421–446.

Chang, Q., Zuo, Z., Chow, M.S., Ho, W.K., 2005. Difference in absorption of the two structurally similar flavonoid glycosides, hyperoside and isoquercitrin, in rats. European Journal of Pharmaceutics and Biopharmaceutics 59, 549–555.

Chen, C.H., Lin, J.Y., Lin, C.N., Hsu, S.Y., 1992. Inhibition of angiotensin-I-converting enzyme by tetrahydroxyxanthones isolated from *Tripterospermum lanceolatum*. Journal of Natural Products 55, 691–695.

De Medeiros, J.M., Macedo, M., Contancia, J.P., Nguyen, C., Cunningham, G., Miles, D.H., 2000. Antithrombin activity of medicinal plants of the Azores. Journal of Ethnopharmacology 72, 157–165.

Emura, K., Yokomizo, A., Toyoshi, T., Moriwaki, M., 2007. Effect of enzymatically modified isoquercitrin in spontaneously hypertensive rats. Journal of Nutritional Science and Vitaminology 53, 68–74.

Enkhmaa, B., Shiwaku, K., Katsube, T., Kitajima, K., Anuurad, E., Yamasaki, M., Yamane, Y., 2005. Mulberry (*Morus alba* L.) leaves and their major flavonol quercetin 3-(6-malonylglucoside) attenuate atherosclerotic lesion development in LDL receptor-deficient mice. Journal of Nutrition 135, 729–734.

Ferreira, R.B.G., Vieira, M.C., Zárete, N.A.H., 2004. Análise de crescimento de *Tropaeolum majus* 'jewel' em função de espaçamentos entre plantas. Revista Brasileira de Plantas Medicinais 7, 57–66.

Ferro, D., 2006. Fitoterapia: conceitos clínicos. Atheneu, São Paulo.

Gallagher, M., Perkovic, V., Chalmers, J., 2006. Diuretics: a modern day treatment option? Nephrology 11, 419–427.

Gaskell, S.J., 1997. Electrospray: principles and practice. Journal of Mass Spectrometry 32, 677–688.

Gasparotto Junior, A., Boffo, M.A., Lourenco, E.L., Stefanello, M.E., Kassuya, C.A., Marques, M.C., 2009. Natriuretic and diuretic effects of *Tropaeolum majus* (Tropaeolaceae) in rats. Journal of Ethnopharmacology 122, 517–522.

Gasparotto Junior, A., Gasparotto, F.M., Boffo, M.A., Lourenco, E.L., Stefanello, M.E., Silva-Santos, J.E., Marques, M.C., Kassuya, C.A., in press. Diuretic and potassium-sparing effect of isoquercitrin – an active flavonoid of *Tropaeolum majus* L. Journal of Ethnopharmacology. doi:10.1016/j.jep.2010.12.009.

Griffiths, D.W., Deighton, N., Birch, A.N., Patrian, B., Baur, R., Stadler, E., 2001. Identification of glucosinolates on the leaf surface of plants from the Cruciferae and other closely related species. Phytochemistry 57, 693–700.

Han, G.Q., Pan, J.X., Li, C.L., Tu, F., 1991. The screening of Chinese traditional drugs by biological assay and the isolation of some active components. International Journal of Chinese Medicine 16, 1–17.

Israili, Z.H., Hall, W.D., 1992. Cough and angioneurotic edema associated with angiotensin-converting enzyme inhibitor therapy. A review of the literature and pathophysiology. Annals of Internal Medicine 117, 234–242.

Jadhav, R.B., Bhatnagar, S.P., Surana, S.J., 2010. Diuretic activity of squamate mistletoe, *Viscum angulatum*. Pharmaceutical Biology 48, 417–421.

Khosla, N., Kalaitzidis, R., Bakris, G.L., 2009. The kidney, hypertension, and remaining challenges. Medical Clinics of North America 93, 697–715.

Kim, B.H., Lee, I.J., Lee, H.Y., Hwang, B.Y., Han, S.B., Kim, Y., 2007. Distinct inhibitory mechanisms of isoquercitrin gallate and its aglycone on zymosan-induced peroxynitrite production in macrophages. Nitric Oxide 17, 134–142.

Kjaer, A., Madsen, J.O., Maeda, Y., 1978. Seed volatiles within the family Tropaeolaceae. Phytochemistry 17, 1285–1287.

Kwon, E.K., Lee, D.Y., Lee, H., Kim, D.O., Baek, N.I., Kim, Y.E., Kim, H.Y., 2010. Flavonoids from the buds of *Rosa damascena* inhibit the activity of 3-hydroxy-3-methylglutaryl-coenzyme a reductase and angiotensin I-converting enzyme. Journal of Agricultural and Food Chemistry 58, 882–886.

Lacaille, D., Franck, U., Wagner, H., 2001. Search for potential angiotensin converting enzyme (ACE)-inhibitors from plants. Phytomedicine 8, 47–52.

Li, J., Wang, Z.W., Zhang, L., Liu, X., Chen, X.H., Bi, K.S., 2008. HPLC analysis and pharmacokinetic study of quercitrin and isoquercitrin in rat plasma after administration of *Hypericum japonicum* thunb. extract. Biomedical Chromatography 22, 374–378.

Li, J.S., Sharifi, A.M., Schiffrin, E.L., 1997. Effect of AT1 angiotensin-receptor blockade on structure and function of small arteries in SHR. Journal of Cardiovascular Pharmacology 30, 75–83.

Lorenzi, H., Matos, F.J.A., 2002. Plantas medicinais no Brasil: nativas e exóticas. Instituto Plantarum, São Paulo.

Lykkesfeldt, J., Moller, B.L., 1993. Synthesis of benzylglucosinolate in *Tropaeolum majus* L. (isothiocyanates as potent enzyme inhibitors). Plant Physiology 102, 609–613.

Mietkiewska, E., Giblin, E.M., Wang, S., Barton, D.L., Dirpaul, J., Brost, J.M., Katavic, V., Taylor, D.C., 2004. Seed-specific heterologous expression of a nasturtium FAE gene in *Arabidopsis* results in a dramatic increase in the proportion of erucic acid. Plant Physiology 136, 2665–2675.

Motoyama, K., Koyama, H., Moriwaki, M., Emura, K., Okuyama, S., Sato, E., Inoue, M., Shioi, A., Nishizawa, Y., 2009. Atheroprotective and plaque-stabilizing effects of enzymatically modified isoquercitrin in atherogenic apoE-deficient mice. Nutrition 25, 421–427.

Nyman, U., Joshi, P., Madsen, L.B., Pedersen, T.B., Pinstrup, M., Rajasekharan, S., George, V., Pushpangadan, P., 1998. Ethnomedical information and in vitro screening for angiotensin-converting enzyme inhibition of plants utilized as traditional medicines in Gujarat, Rajasthan and Kerala (India). Journal of Ethnopharmacology 60, 247–263.

Opie, L.H., 1996. ACE inhibitors in pregnancy—how to avoid the sting in the tail. South African Medical Journal 86, 326–327.

Park, P.J., Je, J.Y., Kim, S.K., 2003. Angiotensin I converting enzyme (ACE) inhibitory activity of hetero-chitooligosaccharides prepared from partially different deacetylated chitosans. Journal of Agricultural and Food Chemistry 51, 4930–4934.

Pechanova, O., Rezzani, R., Babal, P., Bernatova, I., Andriantsitohaina, R., 2006. Beneficial effects of Provinols: cardiovascular system and kidney. Physiological Research 55 (Suppl. 1), S17–S30.

Pfeffer, M.A., Frohlich, E.D., 2006. Improvements in clinical outcomes with the use of angiotensin-converting enzyme inhibitors: cross-fertilization between clinical and basic investigation. AJP-Heart and Circulatory Physiology 291, 2021–2025.

Pierdomenico, S.D., Bucci, A., Lapenna, D., Cuccurullo, F., Mezzetti, A., 2002. Heart rate in hypertensive patients treated with ACE inhibitors and long-acting dihydropyridine calcium antagonists. Journal of Cardiovascular Pharmacology 40, 288–295.

Roesler, R., Catharino, R.R., Malta, L.G.N., Pastore, E.M.G., 2008. Antioxidant activity of *Caryocar brasiliense* (pequi) and characterisation of components by electrospray ionization mass spectrometry. Food Chemistry 110, 711–717.

Sakamoto, T., Chen, C., Lokhandwala, F., 1994. Contribution by bradykinin to the natriuretic response to the angiotensin converting enzyme inhibitor ramiprilat in spontaneously hypertensive rats. Naunyn-Schumiedeberg's Archives of Pharmacology 350, 84–89.

Santos, R.A., Krieger, E.M., Greene, L.J., 1985. An improved fluorometric assay of rat serum and plasma converting enzyme. Hypertension 7, 244–252.

Sarikonda, V.K., Watson, R.E., Opara, O.C., DiPette, D.J., 2009. Experimental animal models of hypertension. Journal of the American Society of Hypertension 3, 158–165.

Schramm, D.D., German, J.B., 1998. Potential effects of flavonoids on the etiology of vascular disease. The Journal of Nutritional Biochemistry 9, 560–566.

Silva, C.G., Raulino, R.J., Cerqueira, D.M., Mannarino, S.C., Pereira, M.D., Panek, A.D., Silva, J.F., Menezes, F.S., Eleutherio, E.C., 2009. In vitro and in vivo determination of antioxidant activity and mode of action of isoquercitrin and *Hyptis fasciculata*. Phytomedicine 16, 761–767.

Somanadhan, B., Varughese, G., Palpu, P., Sreedharan, R., Gudiksen, L., Smitt, U.W., Nyman, U., 1999. An ethnopharmacological survey for potential angiotensin

converting enzyme inhibitors from Indian medicinal plants. Journal of Ethnopharmacology 65, 103–112.
Strittmatter, S.M., Snyder, S.H., 1986. Characterization of angiotensin converting enzyme by [3H] captopril binding. Molecular Pharmacology 29, 142–148.
Vitor, R.F., Mota-Filipe, H., Teixeira, G., Borges, C., Rodrigues, A.I., Teixeira, A., Paulo, A., 2004. Flavonoids of an extract of *Pterospartum tridentatum* showing endothelial protection against oxidative injury. Journal of Ethnopharmacology 93, 363–370.
Wagner, H., Elbl, G., 1992. ACE-inhibitory procyanidins from *Lespedeza capitata*. Planta Medica 58, 297.
Wielanek, M., Urbanek, H., 2006. Enhanced glucotropaeolin production in hairy root cultures of *Tropaeolum majus* L. by combining elicitation and precursor feeding. Plant Cell, Tissue and Organ Culture 86, 177–186.
Williams, B., Poulter, N.R., Brown, M.J., Davis, M., McInnes, G.T., Potter, J.F., Sever, P.S., Thom, S.M., 2004. British Hypertension Society guidelines for hypertension management 2004 (BHS-IV): summary. BMJ 328, 634–640.
Wright, C.I., Van-Buren, L., Kroner, C.I., Koning, M.M., 2007. Herbal medicines as diuretics: a review of the scientific evidence. Journal of Ethnopharmacology 114, 1–31.
Wu, J., Muir, A.D., 2008. Isoflavone content and its potential contribution to the antihypertensive activity in soybean Angiotensin I converting enzyme inhibitory peptides. Journal of Agricultural and Food Chemistry 56, 9899–9904.
Ye, M., Han, J., Chen, H., Zheng, J., Guo, D., 2007. Analysis of phenolic compounds in rhubarbs using liquid chromatography coupled with electrospray ionization mass spectrometry. Journal of The American Society for Mass Spectrometry 18, 82–91.
Yung, L.M., Leung, F.P., Wong, W.T., Tian, X.Y., Yung, L.H., Chen, Z.Y., Yao, X.Q., Huang, Y., 2008. Tea polyphenols benefit vascular function. Inflammopharmacology 16, 230–234.
Zhang, Y., Lee, E.T., Devereux, R.B., Yeh, J., Best, L.G., Fabsitz, R.R., Howard, B.V., 2006. Prehypertension, diabetes, and cardiovascular disease risk in a population-based sample: the Strong Heart Study. Hypertension 47, 410–414.