Antihypertensive Effect of Carica papaya Via a Reduction in ACE Activity and Improved Baroreflex

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Antihypertensive Effect of *Carica papaya* Via a Reduction in ACE Activity and Improved Baroreflex

Authors Girlandia Alexandre Brasil[1], Silas Nascimento Ronchi[1], Andrews Marques do Nascimento[1], Ewelyne Miranda de Lima[1], Wanderson Romão[2,3], Helber Barcellos da Costa[3], Rodrigo Scherer[1], José Aires Ventura[4], Dominik Lenz[1], Nazaré Souza Bissoli[5], Denise Coutinho Endringer[1,2*], Tadeu Uggere de Andrade[1,6*]

Affiliations The affiliations are listed at the end of the article

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Correspondence
Prof. Dr. Tadeu Uggere de Andrade
Universidade Vila Velha
Av. Comissário José Dantas de Melo, n°21, Boa Vista
Vila Velha – ES. CEP 29102–920
Brazil
Phone: + 55 27 34 21 20 01
Fax: + 55 27 34 21 20 00
tadeu.andrade@uvv.br

Correspondence
Prof. Dr. Denise Coutinho Endringer
Universidade Vila Velha
Av. Comissário José Dantas de Melo, n°21, Boa Vista
Vila Velha – ES. CEP 29102–920
Brazil
Phone: + 55 27 34 21 20 01
Fax: + 55 27 34 21 20 00
denise.endringer@uvv.br

Abstract

The aims of this study were to evaluate the antihypertensive effects of the standardised methanolic extract of *Carica papaya*, its angiotensin converting enzyme inhibitory effects *in vivo*, its effect on the baroreflex and serum angiotensin converting enzyme activity, and its chemical composition. The chemical composition of the methanolic extract of *C. papaya* was evaluated by liquid chromatography-mass/mass and mass/mass spectrometry. The angiotensin converting enzyme inhibitory effect was evaluated *in vivo* by Ang I administration. The antihypertensive assay was performed in spontaneously hypertensive rats and Wistar rats that were treated with enalapril (10 mg/kg), the methanolic extract of *C. papaya* (100 mg/kg; twice a day), or vehicle for 30 days. The baroreflex was evaluated through the use of sodium nitroprusside and phenylephrine. Angiotensin converting enzyme activity was measured by ELISA, and cardiac hypertrophy was evaluated by morphometric analysis. The methanolic extract of *C. papaya* was standardised in ferulic acid (203.41 ± 0.02 µg/g), caffeic acid (172.60 ± 0.02 µg/g), gallic acid (145.70 ± 0.02 µg/g), and quercetin (47.11 ± 0.03 µg/g). The flavonoids quercetin, rutin, nicotiflorin, clitorin, and manghaslin were identified in a fraction of the extract. The methanolic extract of *C. papaya* elicited angiotensin converting enzyme inhibitory activity. The antihypertensive effects elicited by the methanolic extract of *C. papaya* were similar to those of enalapril, and the baroreflex sensitivity was normalised in treated spontaneously hypertensive rats. Plasma angiotensin converting enzyme activity and cardiac hypertrophy were also reduced to levels comparable to the enalapril-treated group. These results may be associated with the chemical composition of the methanolic extract of *C. papaya*, and are the first step into the development of a new phytotherapic product which could be used in the treatment of hypertension.

Abbreviations

ACE:	angiotensin converting enzyme
MECP:	methanolic extract of *Carica papaya*
LC-ESI-MS/MS:	liquid chromatography-mass/mass
MS/MS:	mass/mass spectrometry
ANG I:	angiotensin I
SHR:	spontaneously hypertensive rats
RAS:	renin angiotensin system
BRS:	baroreflex sensitivity
WKY:	Wistar-Kyoto
MAP:	mean arterial pressure
HR:	heart rate
SHRC:	SHR control group
SHRE:	SHR treated with enalapril
SHRCP:	SHR treated with *Carica papaya* extract
WHYC:	Wistar-Kyoto control group
WKYE:	Wistar-Kyoto treated with enalapril

Introduction

Several studies have reported cardiovascular effects related to *Carica papaya* L. (Caricaceae – *C. papaya*) [1,2]. The extract of stems [3] or leaves [4] of *C. papaya* showed *in vitro* ACE inhibitory activity. Runnie et al. [5] observed that the methanolic extract of leaves of *C. papaya* elicited vasorelaxant activity on the mesenteric bed and the aorta rings of rats. The papaya juice elicited an antihypertensive effect in the DOCA-salt model [1],

* These two authors contributed equally to this work.

Table 1 ESI(−)-FT-ICR MS and ESI(−)-MS/MS results for the main ions: measured and theoretical *m/z*; mass error (ppm); DBE; and fragments obtained from MS^2 experiments.

$m/z_{measured}$	$m/z_{theoretical}$	$[M-H]^-$	Error[a]	DBE	MS[b]	Flavonoid
593.1519	593.1512	$[C_{27}H_{29}O_{15}-H]$	−1.26	13	593 and 285	Nicotiflorin
609.1468	609.1261	$[C_{27}H_{29}O_{16}-H]$	−1.19	13	609 and 300	Rutin
739.2096	739.2091	$[C_{33}H_{39}O_{19}-H]$	−0.64	14	739, 575, and 284	Clitorin
755.2044	755.2040	$[C_{33}H_{39}O_{20}-H]$	−0.54	14	755 and 300	Manghaslin
793.5147	–	$[C_{33}H_{39}O_{20}+R^1-H]$	–	–	794, 755, 537, and 300	–

[a] Error (ppm) = $[(m/_{measured} - m/z_{theoretical})/m/z_{theoretical}] \times 10^6$; [b] R = 38 Da

and the extracts of *C. papaya* roots diminished increases in MAP in a renovascular model of hypertension [2]. Polyphenolic and flavonoid substances were identified in a methanolic extract of *C. papaya* that showed ACE inhibitory activity [6–8] and antihypertensive effects [9–11].

ACE is a component of the RAS, which is responsible for the long-term control of blood pressure [12,13]. Blood pressure is also controlled by the baroreflex, which is an important short-term reflex that controls "beat to beat" responses [14]. The RAS and baroreflex interact and play a key role in the control of the cardiovascular system [15–17]. Angiotensin II (Ang II), the main vasoconstrictor of the RAS, can reduce BRS by increasing blood pressure and sympathetic regulation [15]. Furthermore, an increase in cardiac hypertrophy can reduce BRS [18] and that hypertrophy can be associated with the local RAS [19].

However, to our knowledge, despite the possible interaction between *C. papaya* and RAS, there are no data in the literature reporting the evaluation of the chronic antihypertensive effects of *C. papaya*. We hypothesise that chronic treatment with this plant reduces the blood pressure of hypertensive animals via inhibition of ACE activity, resulting in the improvement of arterial baroreflex sensitivity.

Therefore, the aim of this study was to evaluate the effects of a chronic administration of a standardised methanolic extract of *C. papaya* leaves on the blood pressure and baroreflex of SHR and to assess the role of ACE inhibition in these effects.

Results

For the polyphenol and flavonoid content, the standardised MECP showed moderated polyphenol and tannin content (47.1 ± 0.6 mg/100 g, 14.3 ± 0.7 mg/100 g, respectively) whereas the flavonoid content was higher (2716.3 ± 78.1 mg/100 g). By, LC-ESI(−)MS/MS was quantified in MECP ferulic acid (203.41 ± 0.02 µg/g), caffeic acid (172.60 ± 0.02 µg/g), gallic acid (145.70 ± 0.02 µg/g), and quercetin (47.11 ± 0.03 µg/g).

The fraction FR4 was submitted to ESI(−)-FT-ICR MS and ESI (−)-FT-ICR MS/MS analyses. The spectrum of FR4 by ESI (−)-FT-ICR-MS (data not shown) showed to be a mixture of flavonoids. The most abundant species present in this spectrum were the ions with *m/z* 739, 755, and 793, and the less abundant species were the ions with *m/z* 408, 555, 593, and 609. Most substances detected by the experiment using ESI(−)directly fusion classified the species as polyphenols that were found in FR4 of MECP.

The most abundant ions (593, 609, 739, and 755) were analysed by ESI(−)-FT-ICR MS/MS and were identified by comparison with previously reported data. These were identified as the flavonoids nicotiflorin, rutin, clitorin, and manghaslin (**Table 1**).

Table 2 Results of the haematological and biochemical analyses after 15 days of treatment with acute doses of 2000 mg/kg of CP.

Parameters	CP	CON
Erythrocytes (million/µL)	11.0 ± 1.0	9.4 ± 0.3
Haemoglobin (g/dL)	13.2 ± 0.7	12.5 ± 1.6
Haematocrit (%)	41.0 ± 2.3	41.6 ± 2.4
VCM	38.8 ± 6.5	49.5 ± 3.0
HCM	10.6 ± 0.2	14.2 ± 2.3
CHCM	32.5 ± 0.3	28.5 ± 3.6
Total protein (%)	6.3 ± 0.1	6.5 ± 0.3
Leukocytes (% × 1000/mm^3)	6.8 ± 0.9	8.5 ± 1.2
ALT (UI/L)	55.2 ± 2.1	53.2 ± 2.9
AST (UI/L)	54.0 ± 2.7	54.4 ± 4.1
Urea (mg/dL)	34.7 ± 1.0	39.3 ± 2.7
Creatinine (mg/dL)	0.60 ± 0.01	0.68 ± 0.13
Troponin I	Negative	negative

Values are expressed as the mean ± S. E.M

For the acute toxicity analyses, no deaths were observed after the administration of an oral dose of MECP of 2000 mg/kg and no macroscopic changes were observed. There were also no changes in the haematological and biochemical parameters of the treated animals compared to the control group (**Table 2**).

After the *in bolus* application of Ang I in the WKY animals, there was a similar increase in the MAP (**Fig. 1**). After the application of captopril or MECP, the administration of Ang I was repeated and no differences were observed for the first dose in both groups (**Fig. 1**).

After the chronic treatment, MECP reduced the MAP of the SHR (**Fig. 2A**), and this reduction was comparable to that obtained with enalapril. MECP or enalapril did not normalise the MAP in the SHRCP ($p < 0.01$) compared to the WKY animals (**Fig. 2A**). The WKY animals did not differ in MAP between the groups. There was no difference in HR between the groups (SHRC: 307 ± 6 bpm, SHRE: 318 ± 20 bpm, SHRCP: 293 ± 6 bpm, WKYC: 287 ± 21 bpm, WKYE: 289 ± 26 bpm, WKYCP: 293 ± 20 bpm).

The analysis of ACE activity after the chronic treatment showed that MECP reduced the ACE activity in the SHR (**Fig. 2B**). There was no difference in ACE activity between the SHRCP and SHRE. The ACE activity in the WKYE and the WKY animals treated with MECP (WKYCP) was lower than in the WKYC.

Also, MECP normalised baroreflex sensitivity in the SHRCP after phenylephrine or sodium nitroprusside applications ($p < 0.01$ compared to SHRC; **Fig. 3A** and **B**). There was no difference between the SHRCP and SHRE animals. The sensitivity of the baroreflex was similar among the normotensive animals (**Fig. 3**).

Treatment normalised the heart weight/body weight (HW/BW) ratio in SHRCP ($p < 0.01$ compared to SHRC; **Fig. 4I**). There was

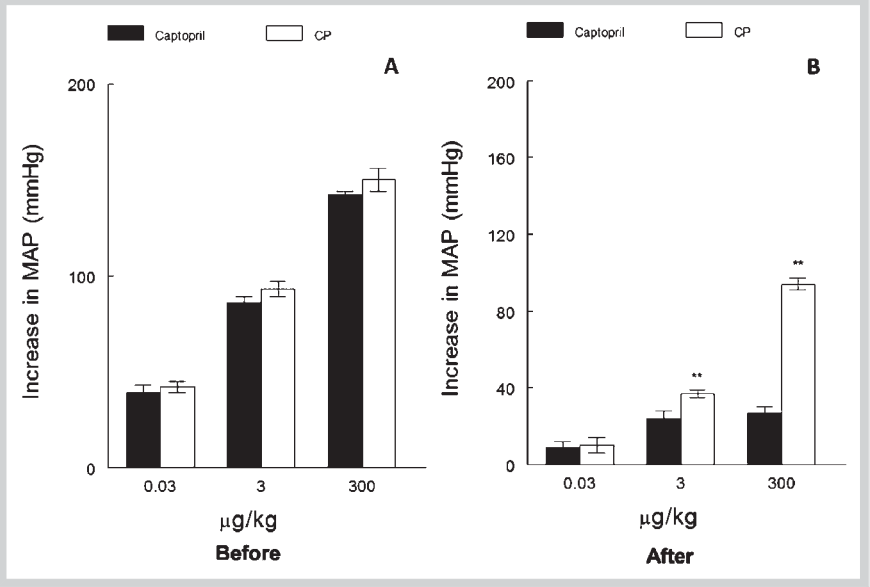

Fig. 1 Increase on MAP by the application of Ang I (0.03, 3, and 300 µg/kg) *in bolus* on WKY animals. **A** Before acute application of captopril (30 mg/kg) or MECP (100 mg/kg). **B** Increase on MAP after acute application of captopril (30 mg/kg) or MECP (100 mg/kg). ** $P < 0.01$ when compared to the increase caused after captopril application.

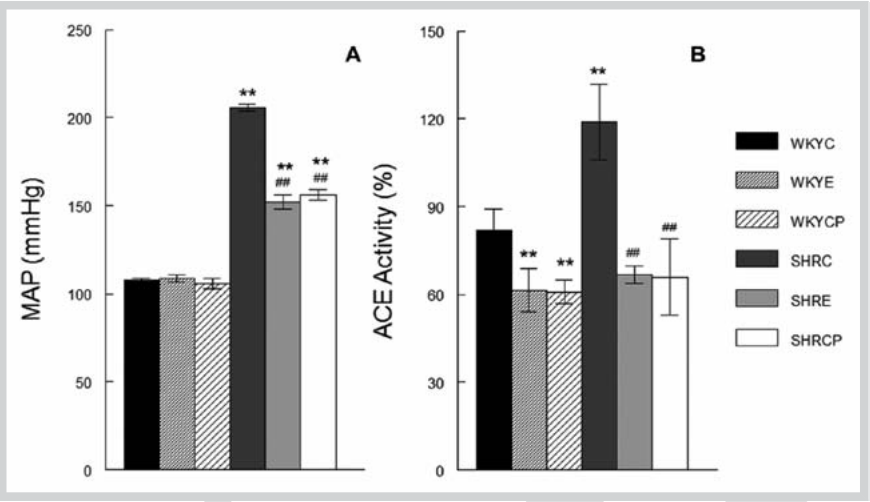

Fig. 2 **A** The MAP of WKYCP, SHRCP, WKYE, SHRE, WKYC, and SHRC for 30 days. ** $P < 0.01$ compared to WKY animals; ## $p < 0.01$ in relation to SHRC. **B** ACE activity in the serum of animals after 30 days of treatment with *C. papaya*, enalapril, or vehicle. ** $P < 0.01$ in relation to WKYC; ## $p < 0.01$ compared to SHRC. The values in both panels are expressed as the mean ± S. E. M.

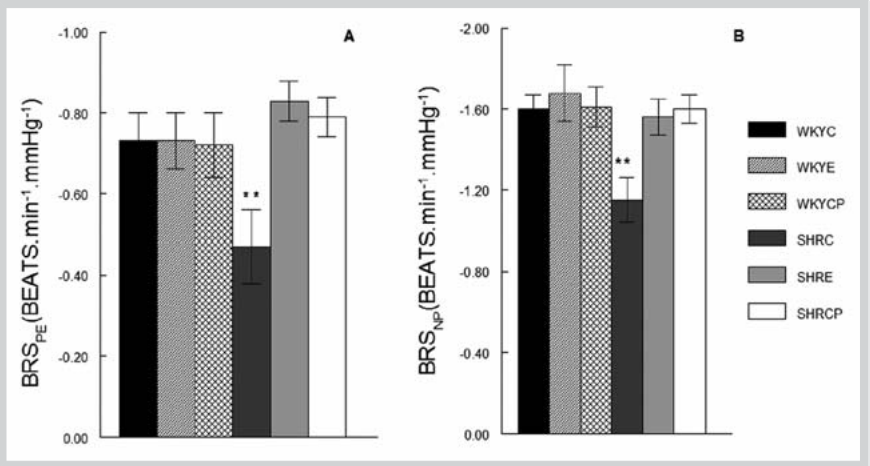

Fig. 3 Result of the gain of arterial baroreflex sensitivity after chronic treatment for WKYCP and SHRCP (100 mg/kg), WKYE and SHRE (10 mg/kg), and WKYC and SHRC (NaCl 0.9 %), twice a day for 30 days, after the administration of phenylephrine (BRS_{PE}; 100 µg/mL; **A**) and sodium nitroprusside (BRS_{NP}: 180 µg/mL; **B**). The values are expressed as the mean ± S. E. M. ** $P < 0.01$ SHRC compared to the other groups.

no difference between the values of the treated SHR and normotensive animals.

The results of morphometric analysis show that in the SHRCP (◉ **Fig. 4**), the number of myocyte nuclei per high-power field and the area of cardiac nuclei were different compared to the

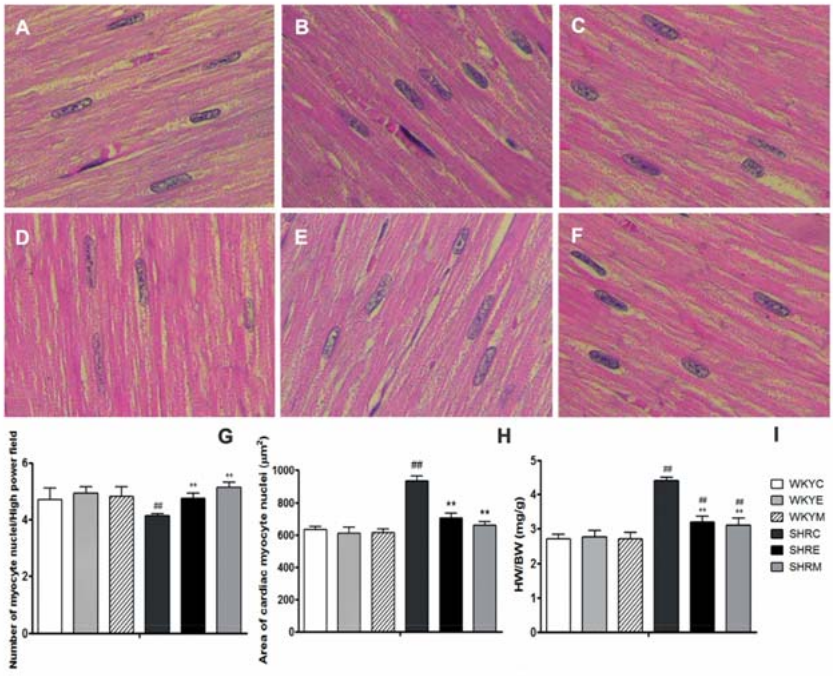

Fig. 4 Histological analysis of the hearts of SHR and WKY animals. The slides were stained with hematoxylin/eosin (H&E), and the analysis was performed at a 400× magnification (**A** WKYC; **B** WKYE; **C** WKYCP; **D** SHRC; **E** SHRE; **F** SHRCP). **G** bar graph corresponding to the number of myocyte nuclei/high-power field. **H** bar graph representing the area of the cardiac myocyte nuclei. **I** HW/BW ratio. Values are expressed as the mean ± S. E. M. ** $P < 0.01$ compared to the WKYC; ## $p < 0.01$ in relation to the SHRC.
(Color figure available online only.)

SHRC ($p < 0.01$), but were not different than those of the normotensive animals. The results of the SHRCP were no different than those of the SHRE.

Body weight gain data demonstrated that the SHRC showed no weight change, while the other groups demonstrated body weight gain [final weight/initial weight (FW/IW) – WKYC: 309 ± 11/237 ± 12 g; WKYE: 304 ± 13/258 ± 7 g; WKYCP: 310 ± 10/251 ± 15 g; SHRC: 269 ± 13/249 ± 15 g; SHRE: 287 ± 11/247 ± 8 g; SHRCP: 261 ± 4/199 ± 9 g].

Discussion

To the best of the authors' knowledge, the present study is the first to demonstrate the chronic antihypertensive effect of *C. papaya* in SHR. The chemical compounds of MECP (polyphenolic and flavonoid compounds) promote cardiac remodelling and improve physiological and structural markers, possibly because of the influence of the components on the RAS, as seen by reducing serum ACE activity of chronically treated animals.

Regarding the chemical composition, our results confirm previously reported data. In addition to alkaloids, tannins, saponins, cardiac glycosides, and anthraquinones, polyphenols and flavonoids were also identified in the leaves of *C. papaya* [20–22]. Additionally, Canini et al. [6] identified the flavonoids quercetin and kaempferol and the caffeic, cumaric, and chlorogenic acids in the leaves of *C. papaya.* With the exception of the cumaric and chlorogenic acids, these compounds were also identified in the present study.

The flavonoid quercetin was identified and quantified in this study in MECP. The flavonoids rutin, maghaslin, clitorin, and nicotiflorin were identified by ESI(−)MS/MS in an extract fraction. The presence of these substances and the content of total flavonoids observed may have contributed to the ACE inhibitory activity. Guerrero et al. [23] demonstrated that flavonoids, such as quercetin, luteolin, and kaempferol, can inhibit ACE activity *in vitro*. Other authors have reported the action of flavonoids in Apple peel extract, which is rich in the flavonoid quercetin, and also has *in vitro* ACE inhibitory activity [9]. Therefore, it seems reasonable to infer that the polyphenolic and flavonoid compounds present in MECP contributed to the *in vivo* ACE inhibitory activity as well as the reduction of the ACE activity after chronic treatment with MECP.

In addition to the reduction in MAP, chronic treatment with MECP reversed both cardiac hypertrophy and the reduction of arterial baroreflex sensitivity. These effects are of great importance because cardiac hypertrophy can be related to both the reduction of cardiac output and to the development of heart failure (reviewed by [24]). Additionally, the baroreflex is extremely important for the beat-to-beat control of MAP and helps reduce beat-to-beat variability [25–27].

Hypertension [28] and the RAS [26,29,30] play important roles in the development of cardiac hypertrophy. Therefore, the reduction of MAP and ACE activity by chronic MECP treatment may contribute to the reversal of cardiac hypertrophy in SHRCP. Several studies have demonstrated that after treatment with ACE inhibitors, there was an improvement of baroreflex in patients with hypertension [31], acute myocardial infarction [32], and heart failure [33]. Therefore, the recovery of arterial baroreflex sensitivity in SHRCP could also be related to ACE inhibition and, consequently, the reduction of MAP.

Minami and Head [18] demonstrated that cardiac hypertrophy plays an important role in baroreflex dysfunction in SHR, and the RAS has a crucial role in the development of cardiac hypertrophy [26,29,30]. Additionally, ACE inhibitors and angiotensin II type 1 receptor antagonists can reverse the development of hypertrophy [34]. Therefore, ACE inhibition by MECP could contribute to the effects on cardiac hypertrophy and baroreflex sensitivity observed in the SHRCP.

The effect of treatment with *C. papaya* on the arterial baroreflex sensitivity is novel, and it appears to be related to those compounds identified in MECP. Monteiro et al. [35], in a study with

isolated quercetin, which is one of the flavonoids identified in MECP, observed a reduction in blood pressure as well as an improvement of baroreflex sensitivity in the SHR treated with this compound.

The acute toxicity assay was performed to estimate the safety of the dose that was used. At a dose of 2000 mg/kg, which is a dose that is one thousand times higher than the dose used to treat the animals, MECP did not present acute toxicity. Subacute toxicity (after 28 days of treatment) of the same dose was also not observed using *C. papaya* [36]. Additionally, no haematological, hepatic, renal, or cardiac marker changes were observed after 2000 mg/kg of MECP administration. Furthermore, the groups treated with MECP, SHRCP, and WKYCP did not show a reduction in body weight after chronic treatment.

In conclusion, MECP has chronic antihypertensive effects in animals, at least in part, via the inhibition of ACE activity, resulting in a reduction in MAP as well as a reversion of the changes in cardiac hypertrophy and baroreflex sensitivity. The presence of polyphenolic and flavonoid compounds in the MECP may contribute to the observed biological effects of this study.

Although wide spectrums of drugs are available for the treatment of hypertension, the cost of its therapy is considerable, mainly for the underdeveloped countries public health systems. The results of this study provide the first step into the development of a new phytotherapic product, which could be used to treat hypertension, even in association with standard pharmacological therapy.

Materials and Methods

Plant material

The cultivar *C. papaya* leaves, Caricaceae, Ruby variety (Rubi INCAPER 511) [37], were provided by Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural and collected from the experimental farm at Sooretama in 2011. A voucher specimen was deposited at herbarium of Universidade Vila Velha (UVVES 2036). The identification was made by Msc. Solange Zanotti Schneider, University Vila Velha. The plant material was dried and ground.

Extraction and fractions

The plant material (160 g) was defatted with hexane (1 L) followed by extraction with methanol (1 L) in a Soxhlet apparatus for 12 h. The liquid extract was then dried in a rotary evaporator under reduced pressure (Fisaton 801) at 40 °C until a residue was formed (25.5 g). This dried extract was evaluated for total polyphenol and flavonoid content [38].

The dried MECP was analysed by LC-ESI-MS/MS, and the *in vitro* ACE inhibitory activity was evaluated.

An aliquot (9.6 g) of MECP was subjected to preliminary fractionation in a silica gel *gateau* (2.3 × 52 cm, 12 g, mesh 0.063–0.2 mm). The gradient elution used was hexane, dichloromethane, dichloromethane : ethyl acetate (1 : 1), ethyl acetate : methanol (1 : 1), and methanol. Five fractions were obtained: hexanic (CPH, 1.6 g), dichloromethanic (CPD, 1.2 g), dichloromethane : ethyl acetate (CPDA, 0.4 g), ethyl acetate : methanol (CPD, 1.9 g), and methanolic (CPAM, 1.9 g), all of which were assessed for the presence of *in vitro* ACE inhibitory activity to determine the active fraction. The *in vitro* ACE inhibitory assay was performed with a colorimetric reaction and assessed with ELISA, as previously described by Endringer et al. [39]. MECP showed significant inhibition of ACE (72.9 ± 3.7 %), and CPDA (100.2 ± 2.0 %), CPAM (92.8 ± 3.7 %), and CPM (76.1 ± 19.1 %) were considered to be active, with an inhibition percentage greater than 50 %. CPAM was selected for refraction according to the biological activity and mass obtained.

CPAM was resuspended in methanol (5 mL), applied to a Sephadex® LH-20 column (1.8 × 24 cm, 6 g, Sigma-Aldrich), and eluted with methanol (Sigma-Aldrich). Fractions were grouped by similarity in TLC, resulting in 15 fractions (F1–F15). FR4 was submitted to ultrahigh resolution and electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometry, ESI (−)-FT-ICR MS.

Identification of compounds by liquid chromatography-mass/mass in the methanolic extract of *Carica papaya*

An Agilent series 1200 HPLC system equipped with an API 3200 (Applied Biosystems) triple quadrupole mass spectrometer was used for chromatographic analyses. The ionisation was performed with an electrospray source in the positive mode (+ 5500 V). All separations were carried out on an Agilent Eclipse plus C18 (150 mm × 4.6 mm i. d., 5 µm) at 35 °C. The mobile phase comprised (A) aqueous formic acid (0.05 %, v/v) and (B) acetonitrile (0.3 mL/min) using a gradient elution of 10–60 % B at 0–8 min, 60–90 % B at 8–12 min, and 90–10 % B at 12–15 min, with a reequilibration time of 6 min. The pressure of the MS nebuliser was 50 psi. The compounds were identified by comparison of the retention time and spectrum with a standard solution (e.g., ferulic acid, caffeic acid, gallic acid, rosmarinic acid, apigenin, and quercetin). Quantification was performed by an external curve with seven points. The validation parameters evaluated were: selectivity, linearity, linear working range, precision, limit of detection, quantification, and practical of quantification. The selectivity was verified from the mass spectra. The repeatability was evaluated with seven consecutive tests, while the intermediate precision was assessed with 15 tests on two different days. The limits of detection and quantification were determined by the signal noise, assessed three and five times, respectively, and the practical limit of quantification was the lower limit of the linear working range [40].

Identification of compounds by electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometry and electrospray ionisation Fourier transform ion cyclotron resonance mass/mass in FR4

An ultrahigh resolution and accuracy mass spectrometer (model 9.4 T Solarix, Bruker Daltonics,), ESI(−)-FT-ICR MS, was used to analyse the FR4 sample. An ESI(−)-FT-ICR spectrum was acquired in the *m/z* region of 200–2000. The sample was dissolved in a methanol/ammonium hydroxide mixture (99.9/0.1 v/v %), resulting in a final concentration of 1 $\mu g \cdot mL^{-1}$. The parameters of the ESI(−) source were performed as previously described [41, 42]. Additionally, tandem mass spectrometry experiments [ESI (−)-FT-ICR MS/MS] were also performed for ions of *m/z* 593, 609, 739, 755, and 793. The spectra were acquired with an isolation window of 1.0 (*m/z* units) and 19–27 V of collision energy (skimmer = − 75 V). All spectra were processed using the Compass Data Analysis (Bruker Daltonics) software package.

Experimental animals

The biological experiments with WKY rats and SHR that were 3 months old and 250–350 g were performed in agreement with the ethical principles of the Brazilian college of animal experimentation [43] and approved by the Ethics, Bioethics and Animal

Welfare of the University Vila Velha committee (UVV) (CEUA-UVV; Protocol 237/2012). The animals were housed in cages with controlled humidity and temperature, a 12-h dark-light cycle and food and water *ad libitum*.

Acute toxicity

The acute toxicity test for the MECP was performed as described in the guide OECD 423 [44]. The animals were treated with a single dose of MECP (2000 mg/kg, orally). Blood samples were collected in tubes for biochemical and haematologic tests. After collection, WKY animals (n = 6) were euthanised with a high dose of anaesthetic and their organs were macroscopically evaluated by a pathologist blinded to the study.

In addition, haematological analyses of total erythrocytes, leucocytes, haemoglobin, protein, and haematocrit as well as the mean corpuscular volume (VCM), corpuscular haemoglobin (HCM), and corpuscular haemoglobin concentration (CHCM) were performed with a haemocytometer (CC550®, Celm). The biochemical analyses of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, and troponin I levels were performed with commercial kits (Bioclin®, Biocon®).

In vivo angiotensin converting enzyme inhibitory activity assay

Animals were anaesthetised with ketamine and xylazine (100/10 mg/kg, Francotar®, Virbac, Xilasin®) and catheterised (femoral artery and vein) according to Andrade et al. [45].

The tests were performed as described by Mangiapane et al. [46], with modifications. The animals were divided into two groups: captopril (C; n = 6) at an intravenous (IV) dose of 30 mg/kg [47] and MECP (n = 6) at an IV dose of 100 mg/kg. Before and after the administration of captopril or MECP, the animals received increasing doses of Ang I (0.03, 3, and 300 µg/kg, IV). The MAP was measured before and after each Ang I administration to obtain the difference of pressure (ΔMAP). The subsequent dose of Ang I was administered after MAP returned to baseline. The inhibition of ACE was evaluated as the difference between ΔMAP for each dose of Ang I before and after the administration of captopril or MECP. The dose of 100 mg/kg of MECP was determined by a dose-effect curve performed to evaluate its acute effect (data not shown).

Determination of antihypertensive effects

WKY and SHR animals were divided into six groups (n = 6, each): WKYC and SHRC controls received saline (NaCl 0.9%, intraperitoneal; IP); WKYE and SHRE controls received enalapril (10 mg/kg, IP; Andrade et al., [48]); and WKYCP and SHRCP groups received MECP (100 mg/kg twice a day, IP). The treatments lasted for 30 days. The animals were weighed at the beginning (initial weight – IW) and at the end of the treatment (final weight – FW) to evaluate the weight gain of the groups.

To randomly allocate the animals in the experimental groups, we performed an indirect blood pressure measurement. To measure the initial systolic blood pressure (SBP) of conscious rats, a tail-cuff manometre was used (IITC Life Science, Inc.) [49]. The mean SBP of the SHR was 207 ± 3 mmHg. The mean of each hypertensive groups was as follows: SHRC: 205 ± 3 mmHg, SHRE: 208 ± 3 mmHg, and SHRCP: 210 ± 4 mmHg. For WKY rats, the mean blood pressure was 108 ± 2, and the values of each group after division were: WKYC: 107 ± 3 mmHg, WKYE: 110 ± 4 mmHg, and WKYCP: 108 ± 3 mmHg.

Haemodynamic and arterial baroreflex evaluation

After treatment, the animals were anaesthetised with ketamine and xylazine (100/10 mg/kg, Francotar®, Virbac; Xilasin®, Syntec) and catheterised [48]. At least 12 h later, the basal MAP and HR of the animals were determined as described by Andrade et al. [45]. After this measurement, the arterial baroreflex was evaluated according to Beutel et al. [50]. In sum, MAP and HR were controlled beat-to-beat in awake animals, and the variation of these parameters was evaluated by the application of phenylephrine (100 µg/mL) and sodium nitroprusside (180 µg/mL) with an infusion pump (EFF311B, Insight, Ribeirão Preto) at a rate of 0.1 mL for 30 s. The relation between the changes in MAP and the respective alteration of the HR was evaluated by regression. The regression coefficient (slope of the curve) expressed as beats per minute (bpm/mmHg) was used as the baroreflex sensitivity index of phenylephrine (BRS_{PE}) or sodium nitroprusside (BRS_{NP}) [51].

Evaluation of angiotensin converting enzyme activity on serum and histological analyses

After the haemodynamic recording, the animals were euthanised by decapitation with a guillotine. Blood was collected, and the serum was separated by centrifugation. The serum was kept at − 80 °C until analysis. The evaluation of ACE activity in serum was performed with a colorimetric assay as described by Franquini et al. [19], with the following modifications; instead of cardiac homogenate, we used 10 µL of serum from the animals that were treated chronically. ACE activity was calculated using the following equation:

$$\% \text{ activity} = (A \times 100)]/B$$

where A is the measured absorbance at 415 nm of rat serum and B is the absorbance of the rabbit lung solution. For determination of cardiac hypertrophy, the hearts were removed, washed with saline, dried with filtre paper, and weighed. The histological analyses for cardiac hypertrophy determination were performed as described by Franquini et al. [19]. The parameters analysed were: number of myocyte nuclei per high-power field, area of the cardiac myocyte nuclei (μm^2), and HW/BW ratio.

Statistical analysis

The results are expressed as the mean ± standard error of the mean (S.E.M.). Data were analysed with a one-way ANOVA followed by Tukey's post hoc test. Tests were adjusted for multiple comparisons, and significance was defined as $p < 0.05$. The software GB-Stat and SlideWrite were used for the analyses and graphic construction, respectively.

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Conflict of Interest

The authors declare no conflict of interest.

Affiliations

[1] Department of Pharmacy, University Vila Velha – UVV, Vila Velha, ES, Brazil
[2] Federal Institute of Education, Science and Technology of the Espírito Santo – IFES, Vila Velha, ES, Brazil
[3] Department of Chemistry, Federal University of Espírito Santo – UFES, Vitoria, ES, Brazil
[4] Capixaba Intitute of Research, Technical Assistance and Rural Extension – Incaper, Vitoria, ES, Brazil
[5] Department of Physiological Sciences, Federal University of Espírito Santo – UFES, Vitoria, ES, Brazil
[6] Department of Pharmaceutical Sciences, Federal University of Espírito Santo – UFES, Vitoria, ES, Brazil

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