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Research Paper

Cardiovascular effects of the essential oil from leaves of *Eugenia sulcata* in spontaneously hypertensive rats

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ABSTRACT

Eugenia sulcata, Spring ex Mart, Myrtaceae, was collected in Restinga de Jurubatiba National Park (RJ, Brazil). Leaves from this species were submitted to hydrodistillation in order to obtain the essential oil. Sesquiterpenes were the main group found (58.2%), and β-caryophyllene was the major constituent (24.6%). Effects of this essential oil on hemodynamic and cardiovascular parameters were evaluated in spontaneously hypertensive rats and Wistar Kyoto rats. The essential oil significantly decreased the systolic ($P<0.05$) and diastolic ($P<0.01$) blood pressure in spontaneously hypertensive rats. It was also observed that the essential oil did not interfere with heart rate of rats. The present study reports for the first time the anti-hypertensive activity of the essential oil extracted from *Eugenia sulcata* leaves.

Keywords: Myrtaceae; *Eugenia sulcata*; Essential oil; Antihypertensive activity.

INTRODUCTION

Cardiovascular disease, including hypertension, remains a major cause for morbidity and mortality around the world (Panchal and Brown, 2013). Several plant species of the family Myrtaceae are used in folk medicine for the treatment of hypertension, including *Pimenta dioica* (L.) Merr (Suárez, et al., 2000), *Psidium guajava* L. (Gutiérrez, 2008) and *Eugenia uniflora* L. (Consolini, 1999). Additionally, essential oils of various species of other families have also been described to present antihypertensive (Barcelos, et al., 2010; Interaminensea, et al., 2013; Interaminensea, et al., 2011; Talpur, et al., 2005) and hypotensive activity (Barbosa-Filho, et al., 2008; De Siqueira, et al., 2010; Lima, et al., 2012b; Menezes, et al., 2010; Moreira, et al., 2010).

Eugenia sulcata Spring ex Mart. belongs to the family Myrtaceae, and is popularly known as “pitanguinha” and “murtinha” (Araújo, et al., 1998; Cruz, et al., 2004). This species is widely distributed in Brazil, occurring from the Amazon to the south of Brazil in virtually all vegetation physiognomies (Sobral, et al., 2013). This species is a common tree of the Atlantic Forest. It may also occur in the wet forest of Central and South America. It grows slowly and may reach 20 m in height (Franceschinelli, et al., 2007). There are few chemical and biological studies reported in the literature regarding this plant species (Lima, et al., 2012a; Santos, et al., 2013).

Our previous work demonstrated the hypotensive activity of *Eugenia sulcata* using male Wistar normotensive rats (Santos, et al., 2013). Here, we evaluate the influence of the essential oil from *Eugenia sulcata* leaves, in terms of hemodynamic and cardiovascular parameters such as systolic blood pressure, diastolic blood pressure, and heart rate in spontaneously hypertensive rats (SHR). To our knowledge, there has been no other study investigating *E. sulcata* as an antihypertensive agent.

MATERIALS AND METHODS

Plant material: Leaves of *Eugenia sulcata* Spring ex Mart., were collected in Restinga de Jurubatiba National Park, Rio de Janeiro State, Brazil, from Clusia scrub vegetation (22°12’57.7”S 41°34’58.5”W) dated April 23, 2011. This species was identified by the botanist Dr. Marcelo Guerra Santos. A voucher specimen was deposited at the herbarium of the Faculdade de Formação de Professores (Universidade do Estado do Rio de Janeiro, Brazil), under the register number RFFP 13.788. This procedure was previously described by Lima et al. (2012a).

Extraction of the essential oil: Leaves of *Eugenia sulcata* were turbolized in distilled water, placed in a 5L round-bottom flask and submitted to hydrodistillation for 4h in a Clevenger-type apparatus (Clevenger, 1928). At the end, the oil was collected and stored at 4°C for further analyses. This procedure was previously described by Lima et al. (2012a).

Gas chromatography/mass spectrometry analysis: The essential oil was analyzed by a GCMS-QP5000 (Shimadzu) gas chromatograph equipped with a mass spectrometer using electron ionization. Gas chromatographic (GC) conditions were as follows: injector temperature, 260°C; FID temperature, 290°C; carrier gas, helium; flow rate, 1 mL/min and split injection with split ratio 1:40. The oven temperature was initially 60°C and then raised to 290°C at a rate of 3°C/min. One microliter of each sample, dissolved in dichloromethane (1:100mg/µl), was injected onto a DB-5 column (i.d. = 0.25mm, length 30m, film thickness=0.25µm). Mass spectrometry (MS) conditions were as follows: voltage 70 eV; scan rate 1 scan/s. Retention indices (RI) were calculated by interpolation to the retention times of a mixture of aliphatic hydrocarbons (C_9-C_{30}) analyzed under the same conditions (Van Den Dool and Kratz, 1963). Identification of the substances was performed by comparison of their retention indices and mass spectra with those reported in literature (Adams, 2007). The MS fragmentation patterns of the compounds were also checked against NIST mass spectra libraries. Quantitative analysis of the chemical constituents was performed by flame ionization gas chromatography (CG/FID), under same conditions of GC/MS analysis. The percentages of these compounds were obtained by the FID peak-area normalization method. This procedure was previously described by Lima et al. (2012a).

Animals: A total of 18 Male Wistar Kyoto rats (WKY) and 18 spontaneously hypertensive rats (SHR), 36 rats were divided into six groups of six rats each, weighing 200-250g, 12 weeks old, were purchased from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS) and were maintained at the Laboratory of Cardiovascular Physiology, Federal University of Pampa, campus Uruguaiana, RS, Brazil, used after acclimatization for 1 week. They were kept under conditions of constant temperature (20-25°C) with an artificial 12h light/dark cycle; all rats received food and water ad libitum. The experimental protocol was carried out according to the COBEA guidelines (Brazilian College of Animal Experimentation) and was approved by the Animal Ethics Committee of the Federal University of Pampa (n° 31/2012).

Experimental groups: Rats were divided into six experimental groups of six rats each. Two control groups of WKY rats and SHR rats received physiological saline solution. Two vehicle groups of WKY rats and SHR rats received only the vehicle (sunflower oil). Two treated groups of WKY rats and SHR rats received the essential oil diluted in the vehicle. Rats were treated for 30 days with daily dose injections, (10 mg/kg, *i.p.*), in a final volume of 200µl.

Blood pressure and heart rate: To measure the blood pressure and heart rate, rats were anesthetized with sodium thiopental (Tiopental®, 40mg/kg, *i.p.*) and a polyethylene catheter (PE 50, Clay-Adams) filled with heparinized saline (100IU/ml; Cristália®) was inserted into the right carotid artery. The arterial catheter was connected to a pre-calibrated pressure transducer (TSD 104A) equipped with an analog-to-digital converter board (MP150 Biopac Systems, Inc., CA). The recording of blood pressure (systolic, diastolic) and heart rate was performed after hemodynamic parameters had stabilized (30min).

Statistical analysis: Blood pressure and heart rate values were expressed as MEAN ± S.E.M. The results were analyzed using one-way ANOVA followed by Tukey's test. Differences were considered statistically significant when $P<0.05$.

RESULTS

Chemical analysis of the essential oil from *Eugenia sulcata* leaves revealed the presence of 22 substances. Sesquiterpenes were the main group, corresponding to 58.2% of the relative composition of this essential oil. β-caryophyllene was the major constituent found, corresponding to 24.6% of the total relative composition. The monoterpenes α-pinene and β-pinene were also found in large quantities, corresponding to 17.2% and 10.9 of the oil, respectively.

Table-1 shows the effect of the essential oil on systolic blood pressure of studied rats. In normotensive rats (WYK), there were no significant differences in systolic blood pressure between the treated group (110.73±2.24mmHg) and the control group (106.76±3.18mmHg) and vehicle group (107.85±5.96mmHg). In spontaneously hypertensive rats (SHR), there were no differences in systolic blood pressure between the control group (153.37±5.71mmHg) and the vehicle group (150.14±5.36mmHg); however, the essential oil from *Eugenia sulcata* leaves significantly decreased systolic blood pressure of SH rats in the treated group (126.15± 5.36mmHg) at $P<0.05$.

Table-2 shows the effect of essential oil on diastolic blood pressure (mmHg) of studied rats. In normotensive rats (WYK), there were also no significant differences in diastolic blood pressure in the treated group (72.42±3.73mmHg) than the control (66.41±3.97mmHg) and vehicle (sunflower oil) (69.37±2.91mmHg)

groups. In spontaneously hypertensive rats (SHR), there were no differences in diastolic blood pressure of control (109.63±5.26mmHg) than vehicle (105.41±3.19mmHg) group; however, essential oil from *E. sulcata* leaves decreased the diastolic blood pressure of treated rats by 20% (85.19±4.74mmHg), at $P<0.01$.

Table-3 shows that the essential oil from *Eugenia sulcata* leaves has no effects on heart rate (bpm) of rats from the different experimental groups studied whether they are normotensive (WYK) or hypertensive (SHR). Normal heart rate was maintained in all rats of experimental groups studied.

DISCUSSION

Rats are widely used to mimic human disease states, especially cardiovascular and endocrine diseases (Chan, et al., 2011). The spontaneously hypertensive rat (SHR) is the most commonly used rat model of human hypertension (Arrieta, et al., 2004; Hwang, et al., 2011; Panchal and Brown, 2013). We used this animal model and the WKY rats as a normotensive control group to study the cardiovascular effects of the essential oil from *Eugenia sulcata* leaves.

Our results suggest that the anthihypertensive action of this essential oil may be partially associated with the sesquiterpene β-caryophyllene and the monotepenes α-pinene and β-pinene (Bigliani, et al., 2012). The sesquiterpene β-caryophyllene which is recognized as a Ca^{2+} channel blocker and can induce a significant decrease in blood pressure, probably due to negative inotropic action (Bigliani, et al., 2012). The monoterpenes α-pinene and β-pinene also are able to decrease blood pressure, but accompanied by bradycardia (Santos, et al., 2011). However, the absence of any effect on heart rate suggests that the possible participation of other constituents from the essential oil in the mediation of cardiovascular effects cannot be excluded.

Since essential oils can be part of an integrative approach for managing hypertension (Walsh, et al., 2011), the evaluation of the antihypertensive effects of species rich in these volatile complex mixtures is required. Present work shows that essential oil from *E. sulcata* leaves significantly decreased both systolic ($P<0.05$) and diastolic ($P<0.01$) blood pressure of spontaneously hypertensive rats, while no effect on heart rate was detected. To our knowledge, no previous study regarding the investigation of *E. sulcata* as an antihypertensive agent. It also contributes to the knowledge about species from Restinga de Jurubatiba National Park, especially regarding the biological activities of essential oils from *Myrtaceae* species.

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Table-1: Mean systolic blood pressure (mmHg) in rats groups treated with the essential oil from *Eugenia sulcata* leaves (10 mg/kg, *i.p*.) for 30 days.

Groups	Mean systolic blood pressure (mmHg)	
	WKY	SHR
Control	106.76±3.18	153.37±5.71
Vehicle	107.85±5.96	150.14±5.36
Treated	110.73±2.24	126.15± 5.36*

- Results are MEAN ± S.E.M (n = 6). * $P<0.05$ vs. control and vehicle.
- WKY: Wistar Kyoto rats, SHR: spontaneously hypertensive rats.

Table-2: Mean diastolic blood pressure (mmHg) in rats groups treated with the essential oil from *Eugenia sulcata* leaves (10 mg/kg, *i.p.*) for 30 days.

Groups	Mean diastolic blood pressure (mmHg)	
	WKY	SHR
Control	66.41±3.97	109.63±5.26
Vehicle	69.37±2.91	105.41±3.19
Treated	72.42±3.73	85.19±4.74*

- Foot notes are same as given in table-1.

Table-3: Mean heart rate (bpm) in rats treated with the essential oil from *Eugenia sulcata* leaves (10 mg/kg, *i.p.*) for 30 days.

Groups	Mean heart rate (bpm)	
	WKY	SHR
Control	273.18±30.37	336.11±28.55
Vehicle	295.52±28.27	330.33±22.71
Treated	289.50±42.80	325.03±34.70

- Foot notes are same as given in table-1.

