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Antihypertensive effect of *Lepechinia caulescens* extract on spontaneously hypertensive rats

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Abstract

The aim of the present study was to examine the antihypertensive effect of methanolic extract from *Lepechinia caulescens* (MELc) and to determine the thoracic aorta reactivity after long-term treatment with MELc. Results showed that MELc at 38 and 120 mg/Kg induced a significant decrease of heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) in comparison with control and similar than captopril (30 mg/Kg). Also, MELc (120 mg/Kg) induced a long-term antihypertensive activity when still down SBP and DBP from fifth day until the end of experiment. Vascular reactivity of vessels from extract-treated animals was improved when were stimulated with carbachol and sodium nitroprusside. However, treatment with noradrenaline enhanced contractile response on these preparations. In conclusion, MELc produced significant antihypertensive and bradycardic effects that may be related with an activation of NO/cGMP pathway.

Keywords: Antihypertensive agent; Lamiaceae; *Lepechinia caulescens*; Ursolic acid; Oleanolic acid; SHR rats

Introduction

The World Health Organization published in 2007 its *Guidelines for Assessment and Management of Cardiovascular Risk,* where reports an estimated of 58 million deaths globally in 2005, from them 30% was produced by Cardiovascular diseases (CVD) (WHO, 2011). CVD are most prevalent causes of death in Western population so far. Moreover, hyp-

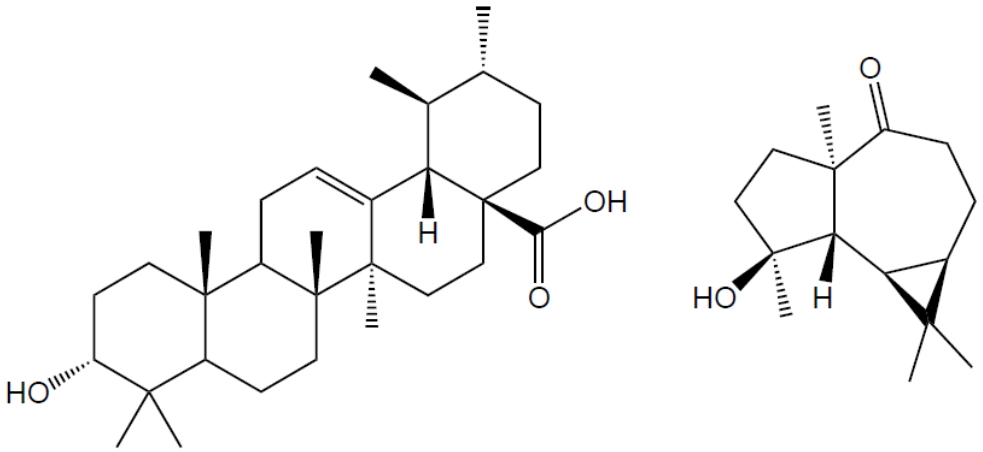

Figure 1. Chemical structure of Ursolic Acid (Left) and Spathulenol (Right).

ertension is one of the most prevalent causes of CVD by impaired vascular relaxation process due to appearance of endothelial dysfunction and oxidative stress (Endemann and Schiffrin, 2004). Therapeutic strategies to combat the consequent damage to the vascular endothelium are generally aimed at modulating the molecular and biochemical mechanisms underlying this dysfunction (Navarrete-Vázquez et al., 2010). One approach to treat the affected endothelium involves improving endothelium-dependent vasodilatation, which is mediated by augmenting the influence of endothelial protective factors (prostacyclin and nitric oxide). Therefore, there are a lot of compounds with NO-release stimulation properties that produce significant relaxant effect in vessels, such as tilianin, naringenin, discretamine, astragaloside IV, galangin, epigallocatechin gallate, *inter alia* (Hernández-Abreu et al., 2009; Morello et al., 2006; Sánchez-Salgado et al., 2010; Silva et al., 2009; Kim et al., 2007; Zhang et al., 2007). In this context, it is well known that the study of medicinal plant species had allowed the isolation of several agents used as leads for the development of new therapeutic drugs (Gurib-Fakim, 2006). Although there is available a low-cost therapy, the Mexican folk medicine policies promote the use of medicinal plants for the treatment of different diseases, and some herbal medicines are real choices for treatment of hypertension (Aguilar et al., 1994; Monroy-Ortiz and Castillo-España, 2007). Consequently, *Lepechinia caulescens* (Ortega) Epling (Lamiaceae) has been used in traditional medicine of Morelos State, Mexico, for the treatment of diabetes, hypertension and related diseases (Monroy-Ortiz and Castillo-España, 2007). However, it has not been widely studied to probe its medicinal uses. Some preliminary pharmacological investigations show that methanolic extract of *L. caulescens* (MELc) and one of its metabolites, ursolic acid (UA) (Figure 1), exert vasorelaxant action on rat aorta rings (Aguirre-Crespo et al., 2005). Furthermore, it was established that their vasorelaxant mechanism is through nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway (Aguirre-Crespo et al., 2006). In addition, other reports showed that this extract was able to induce a spasmolytic effect on spontaneously contraction of rat ileum strips through calcium channel blockade and NO/cGMP pathway (Estrada-Soto et al., 2007).

In this context, the aim of current work was to evaluate the short-term *in vivo* cardiovascular effects of MELc on spontaneously hypertensive rats (SHR). Additionally, long-term cardiovascular effect of MELc was evaluated on same model and vascular reactivity of rat aorta preparations was determined.

Materials and methods

Chemicals and drugs

Carbamylcholine (carbachol), noradrenaline HCl (NA), ursolic acid (UA), captopril and sodium nitroprusside (SNP) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Other reagents were analytical grade from local sources. To carry out the experiments, extracts were dissolved in isotonic salt solution (SS).

Plant material

L. caulescens was collected in February 2004. Briefly, plant material was obtained from its natural habitat (19°05´35.78" N; 98°56´41.99" W; 2 876 m; ©2005Google Earth) and was collected and identified by Dr. P. Castillo-España. A voucher specimen (20386) has been deposited at ''Centro de Educación Ambiental e Investigación Sierra de Huatla'' HUMO-Herbarium, Cuernavaca, Morelos, Mexico.

Preparation of extracts

MELc was obtained as previously described (Aguirre-Crespo et al., 2005). Briefly, air-dried plant material (100 g of aerial parts) was ground into powder and extracted exhaustively by maceration at room temperature with methanol (MeOH; 1 L), which yielded 12.2 g of extract (12.2 %). For *in vivo* experiments, MELc was dissolved in Tween 80 (2%), brought to the chosen volume with sterile isotonic saline solution (vehicle) and sonicated just before use.

Animals

Male spontaneously hypertensive rats [SHR: 250-300 g; 408.5 ± 4.1 bpm for hearth rate (HR); 152.6 ± 2.4 and 122.1 ± 1.9 mmHg for systolic blood pressure (SBP) and diastolic blood pressure (DBP), respectively] were used for this study. Animals were maintained under standard laboratory conditions with free access to food and water. All animal procedures were carried out in accordance with our Federal Regulations for Animal Experimentation and Care (Ministry of Agriculture, NOM-062-ZOO-1999, Mexico) and were approved by the Institutional Animal Care and Use Committee.

Antihypertensive effect of MELc

Acute determination

SHR rats were used in the experiments. All experiments were carried out using six animals per group. Doses used were 38.4 and 120 mg/Kg for MELc and 30 mg/Kg for captopril (used as antihypertensive reference drug, suspended in 0.05% of Tween 80 in SS); test samples were administered by orally intragastric route. Control rats received vehicle (SS) at the same volume (0.5ml/100g). SBP, DBP and HR were measured at 0, 2, 4 and 6 hrs after treatment using a non-invasive tail-cuff method (®Letica, PanLab, Barcelona, Spain).

Sub-acute experimental model

SHR rats were allotted into two groups, untreated control (SS) and MELc group. All experiments were carried out using six animals per group. Treated MELc extract group received 120 mg/kg body weight/day for 10 days. Every day, SBP, DBP and HR were measured before administration of samples test.

In vitro experiments

At the end of sub-acute experiment (MELc and SS groups), thoracic aortic rings were obtained and prepared from SHR rats. The animals were sacrificed by cervical dislocation. Tissue segments were dissected-out, cleaned and placed in organ baths containing warmed (37°C) and oxygenated (O_2:CO_2, 19:1) Krebs solution (10 mL; composition mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; EDTA, 0.026; glucose, 11.1, pH 7.4). Changes in tension were recorded by Grass-FT03 force transducers (Astromed, West Warwick, RI, USA), connected to a MP100 analyzer (®BIOPAC Instruments, Santa Barbara, CA, USA) as previously described (Aguirre-Crespo et al., 2006). All tissues (3-5 mm) were mounted by stainless steel hooks under an optimal tension of 3 g in organ baths with Krebs solution. After equilibration (30 min), rings were contracted with NA (0.1 μM) and washed every 30 min for 2 hrs. The absence of endothelium was confirmed by the lack of a relaxing response to carbachol (1 μM). Thus, cumulative-response curves to carbachol (0.1 nM to 1 μM, endothelium-intact rings), SNP (1 nM to 0.32 μM, endothelium-denuded rings) and NA (0.1 nM to 0.32 μM, endothelium-denuded rings) were recorded for each ring. The effects of agents were determined by comparing the muscular tone of the contraction before and after addition of the test materials. Muscular tone was calculated from the tracings, using Acknowledge software (BIOPAC® system).

Data analysis

Results are expressed as the mean of six experiments ± S.E.M. Concentration-Response Curves (CRC) were plotted and fitted by specific software (ORIGIN® 8.0). The time-course curves represent either number of measurements of HR, SBP and DBP versus time (h.). The statistical significance ($p<0.05$) of differences between means was assessed by an analysis of variance (ANOVA).

Results

MELc (120 mg/Kg) showed important reduction of HR than SS group (Figure 2a) but was less potent compared to captopril treated group ($p<0.05$). However, basal values of HR were recovered at 24 h after administration (data not showed). In addition, MELc at 38 and 120 mg/Kg also induced significantly reduction of SBP (Figure 2b) and DBP (Fig. 2c) compar-ed with control group (vehicle). Moreover, it was more potent than positive control group ($p<0.05$). In fact, both doses of MELc kept down SBP and DBP levels after 24 hrs (data not showed). It is important to mention that doses of 38 mg/Kg of MELc were selected in order to compare the effect of the test sample with the dose used for captopril at 30 mg/kg (50 and 100 mg/Kg are therapeutic doses used for the treatment of hypertension), also 120 mg/Kg of MELc were established using a median logarithm above ratio based on first dose.

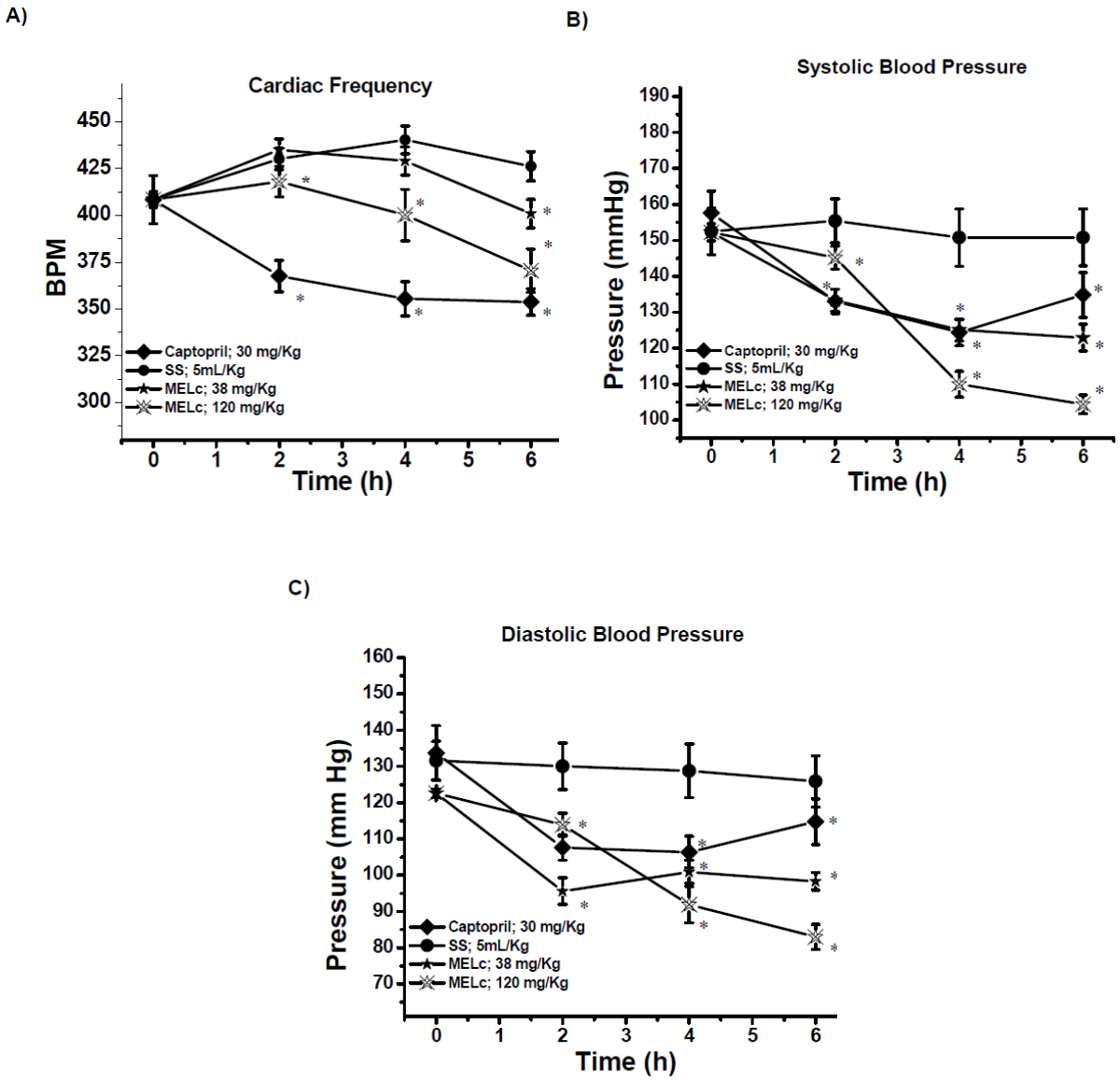

Figure 2. Effect of intragastric administration of MELc at doses of 38.4 and 120 mg/Kg and captopril (30 mg/Kg) on (**a**) heart rate, (**b**) systolic blood pressure and (**c**) diastolic blood pressure in SHR (n=6; *p< 0.01, MELc vs SS).

Orally sub-acute treatment with MELc (120 mg/Kg, 10 days) can regulate the rise of diastolic and systolic blood pressures in SHR without changes on HR (Figure 3). After 6 days of treatment, MELc showed a significant reduction on SBP and DBP than control group (112.6±3.3 and 90.6±3.6 *vs.* 131.3±4.0 and 107.8±2.5 mm Hg, for SBP and DBP, respectively). After evaluation of sub-acute effect of MELc in SHR, thoracic aortas were dissected out from both experimental groups and concentration-response curves for sodium nitroprusside (SNP), carbachol and noradrenaline (NA) were constructed. SNP and carbachol relaxed curves (Figure 4a and 4b) were shifted to the left on aortic rings obtained from rats treated with MELc (CI_{50}: 6.3 ± 0.03 nM *vs.* 0.398 ± 0.1 µM for NPS; and 3.98 ± 0,2 nM *vs.* 0.398 ± 0.1 M for carbachol) compared to the control group, respectively. Maximum effect of SNP-induced relaxation was unaffected in MELc group (Emax: 99.1±0.7 *vs.* 97.01±2.3 % of relaxation); however, the maximal relaxation induced by carbachol in the extract group was

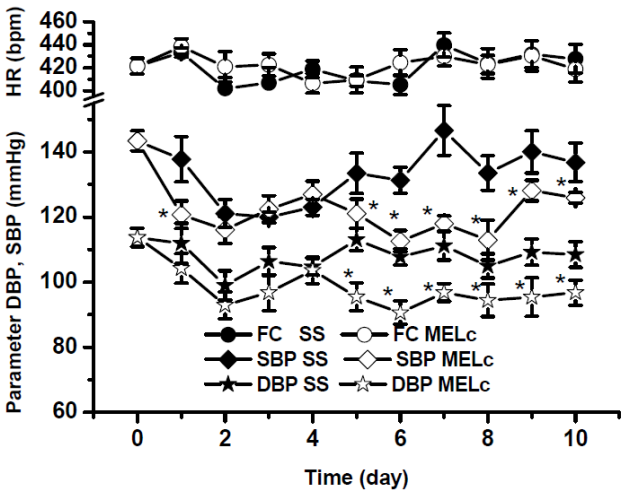

Figure 3. Sub-acute antihypertensive effect of MELc (120 mg/Kg) in SHR (n=6; **p < 0.01, SS vs. MELc).

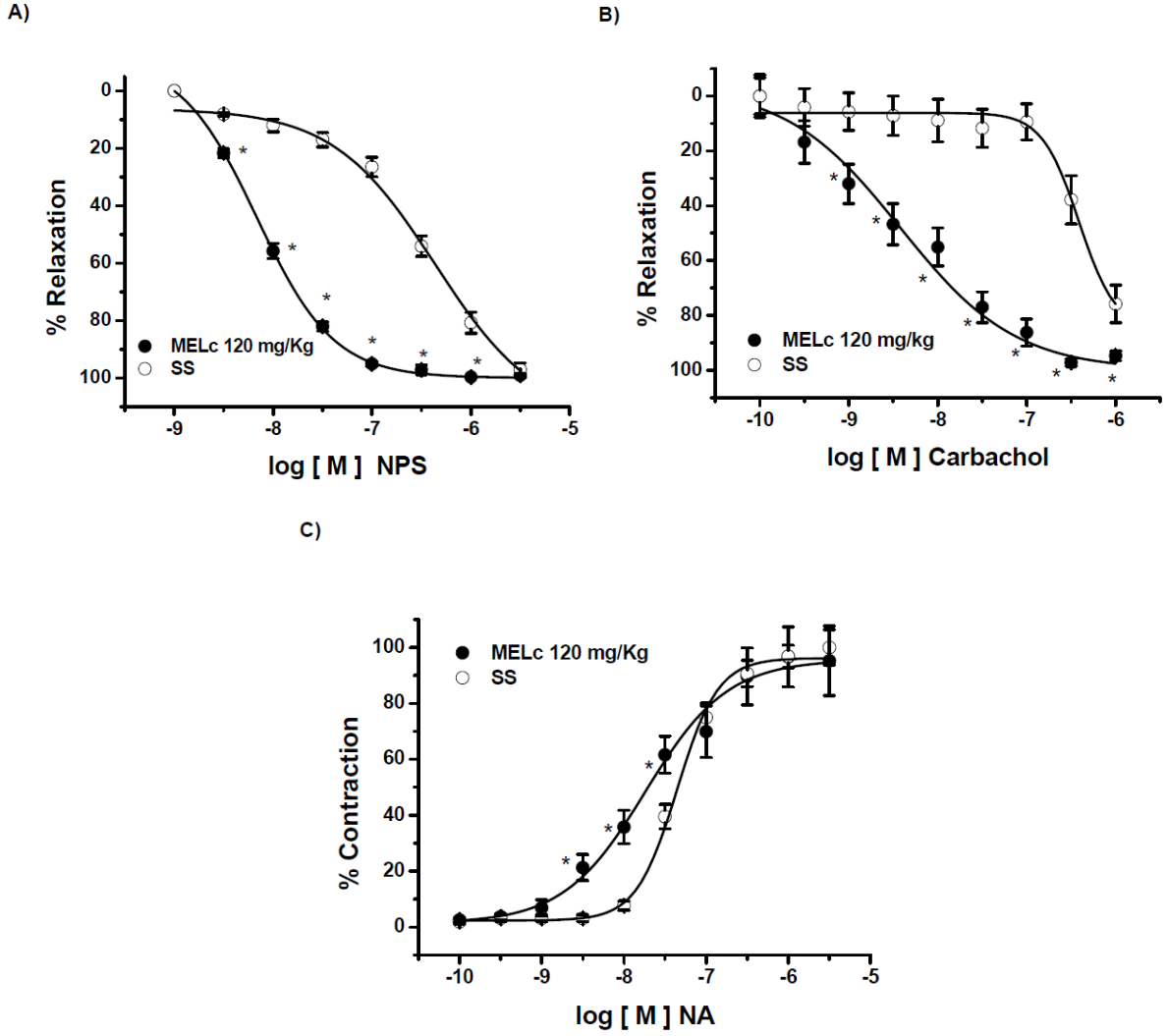

Figure 4. Concentration-Response Curves to noradrenaline (NA), carbachol and sodium nitroprusside (SNP) in control conditions (SS) and treated with MELc (120 mg/Kg/day, 10 days), in SHR. (n= 4; **p < 0.01, SS vs. MELc).

approximately 20% more than control group (94.72 ± 1.89 *vs.* 75.86 ± 5.93 %). Finally, there was no significant difference in the maximal contraction to NA between two groups (Figure 4c; E_{max}: 1.7 ± 0.2 *vs.* 1.9 ± 0.1 g), but the concentration-response curve was significantly shifted to the left (IC_{50}: 19.95 ± 0.1 *vs.* 39.81 ± 0.05 M).

Discussion

Lepechinia caulescens is an herb used in traditional medicine of Mexico for the treatment of Diabetes mellitus, diarrhea and high blood pressure, through daily consumption of oral beverages or teas (Monroy-Ortiz and Castillo-España, 2007). Previous studies carried out at our laboratory have demonstrated that MELc and ursolic acid have a significantly vasorelaxant effect in endothelium-intact rat aorta rings, through release of NO to vascular smooth muscle (Aguirre-Crespo et al., 2005; 2006). Thus, in order to associate the vasorelaxant effect of MELc with the possible cardiovascular effect in hypertensive animals, we employed SHR model to determine its pharmacological effect in acute (6 hours) and subacute (10 days) test. In this context, MELc showed acute cardiovascular effect in a preliminary dose- dependent manner, and this effect is in agreement with previous *in vitro* results. Moreover, MELc was capable to control hypertension from 6^{th} day of treatment to 10^{th}, which allows us to probe its efficacy as antihypertensive agent. Taken it together, results suggest that MELc induced its antihypertensive effect by vasodilator properties through an activation of NO pathway (Aguirre-Crespo et al., 2005). Also, the exploration of the reactivity of aortic rings from both groups suggested that continuous administration of MELc could induce an augment in the efficiency of systems implicated in relaxation derived from endothelial and smooth muscle cells. So, it was reported that aqueous extract of *Salvia miltiorrhiza* (Lamiaceae) and UA increased eNOs expression in EA.hy 926 cells from native human umbilical vein endothelial cells (HUVEC). In addition, they enhanced the bioactive NO and cGMP production reduced NADPH oxidase subunit Nox4 expression and suppressed the production of reactive oxygen species (ROS) in human endothelial cells (Steinkamp-Fenske et al., 2007a; Steinkamp-Fenske et al., 2007b). Thus, MELc might change blood pressure by the modification of expression of the enzyms related with NO/cGMP pathway and by a possible restoration of the functional activity of endothelium and smooth muscle cells. Finally, displacement of concentration-response curves to carbachol and SNP in aortic rings from animals treated with the extracts could be related with UA or oleanolic acid (OA) content in MELc by possible eNOS upregulation, enhancement of bioactive NO production and/or by reduction of the oxidative stress on endothelium cells from SHR, by down regulating of Nox4 expression (Steinkamp-Fenske et al., 2007a; Steinkamp-Fenske et al., 2007b).

UA is one of the main active components of *L.caulescens* that induce vasorelaxation in a concentration- and endothelium-dependent manner (Aguirre-Crespo et al., 2006). However, some other metabolites have been isolated from *L. caulescens* and were reported as possible smooth muscle relaxant agents as terpinen-4-ol, salvigenin and spathulenol (Figure 1) (Lahlou et al., 2002; Perez-Hernandez et al., 2007; Uydeş-Doğan et al., 2005). Thus, we cannot discard their possible participation in cardiovascular MELc's properties. However, early studies reported that triterpenoids such as oleanolic acid, erythrodiol, maslinic acid or uvaol produced a vasorelaxant activity in aortic rat rings of Wistar and SHR rats (Rodriguez-Rodriguez et al., 2004; 2006). So, amelioration in blood pressure of SHR rats could be related with the presence of triterpenoids in plant material of *L. caulescens*. Some reports showed that these molecules (60 mg/Kg, p.w. 6 weeks) exert antihyperlipidemic and antioxidant

effects in Dalh salt-sensitive rats (Somova et al., 2003), and supports the idea that sub-acute treatment could be involved in prevention of hypertension. Another research work indicated that UA and OA protect to isoproterenol-induced myocardial ischemia (Senthil et al., 2007). Further experiments are necessary in order to corroborate or discard the important participation of NO system in anti-hypertensive effect induced by MELc and participation of UA and OA.

In conclusion, MELc produced significant antihypertensive and vasodilatory effects may be by an activation of NO/cGMP pathway that could be related with the presence of ursolic and oleanolic acids.

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Conflict of interests

The authors declared no conflict of interest.

References

Aguilar A, Camacho JR, Chino S, Jácquez P, López ME. (1994). Herbario medicinal del Instituto Mexicano del Seguro Social. México: IMSS.

Aguirre-Crespo FJ, Castillo-España P, Villalobos-Molina R, López-Guerrero JJ, Estrada-Soto S. (2005). Vasorelaxant effect of Mexican medicinal plants on isolated rat aorta. *Pharmaceutical Biology* 43, 540-546.

Aguirre-Crespo FJ, Vergara-Galicia J, Villalobos-Molina R, Lopez-Guerrero JJ, Navarrete-Vazquez G, Estrada-Soto S (2006). Ursolic acid mediates the vasorelaxant activity of *Lepechinia caulescens* via NO release in isolated rat thoracic aorta. *Life Science* 79, 1062-1068.

Endemann DH, Schiffrin EL. (2004). Endothelial dysfunction. *Journal of the American Society of Nephrology* 15, 1983-1992.

Estrada-Soto S, Rodríguez-Avilez A, Castañeda-Avila C, Castillo-España P, Navarrete-Vázquez G, Hernández L, Aguirre-Crespo F. (2007). Spasmolytic action of *Lepechinia caulescens* is through calcium channel blockade and NO release. *Journal of Ethnopharmacology* 114, 364-370.

Gurib-Fakim A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine* 27, 1–93.

Hernández-Abreu O, Castillo-España P, León-Rivera I, Ibarra-Barajas M, Villalobos-Molina R, González-Christen J, Vergara-Galicia J, Estrada-Soto S. (2009). Antihypertensive and vasorelaxant effects of tilianin isolated from *Agastache mexicana* are mediated by NO/cGMP pathway and potassium channel opening. *Biochemical Pharmacol*ogy 78, 54–61.

Kim JA, Formoso G, Li Y, Potenza MA, Marasciulo FL, Montagnani M, Quon MJ. (2007). Epigallocatechin gallate, a green tea polyphenol, mediates NO-dependent vasodilation using signaling pathways in vascular endothelium requiring reactive oxygen species and Fyn. *Journal of Biological Chemistry* 282, 13736–13745.

Lahlou S, Galindo CA, Leal-Cardoso JH, Fonteles MC, Duarte GP. (2002). Cardiovascular effects of the essential oil of *Alpinia zerumbet* leaves and its main constituent, Terpinen-4-ol, in rats: role of the autonomic nervous system. *Planta Medica* 68, 1097-102.

Monroy-Ortiz C, Castillo-España E. (2007). Plantas medicinales utilizadas en el Estado de Morelos, 2nd ed., Cuernavaca, Morelos, México: Universidad Autónoma del Estado de Morelos; pp. 154–156.

Morello S, Vellecco V, Alfieri A, Mascolo N, Cicala C. (2006). Vasorelaxant effect of the flavonoid galangin on isolated rat thoracic aorta. *Life Science* 78, 825–830.

Navarrete-Vázquez G, Hidalgo-Figueroa S, Torres-Piedra M, Vergara-Galicia J, Rivera-Leyva JC, Estrada-Soto S, León-Rivera I, Aguilar-Guardarrama B, Gómez MY, Villalobos-Molina R, Ibarra-Barajas M. (2010). Synthesis, vasorelaxant activity and antihypertensive effect of benzo[d]imidazole derivatives. *Bioorganic and Medicinal Chemistry* 18, 3985–3991.

Perez-Hernandez N, Ponce-Monter H, Medina JA, Joseph-Nathan P. (2007). Spasmolytic effect of constituents from *Lepechinia caulescens* on rat uterus. *Journal of Ethnopharmacology* 115, 30-35.

Rodriguez-Rodriguez R, Herrera MD, Perona JS, Ruiz-Gutiérrez V. (2004). Potential vasorelaxant effects of oleanolic acid and erythrodiol, two triterpenoids contained in 'orujo' olive oil, on rat aorta. *British Journal of Nutrition* 92, 635-642.

Rodriguez-Rodriguez R, Perona JS, Herrera MD, Ruiz-Gutierrez V. (2006). Triterpenic compounds from "orujo" olive oil elicit vasorelaxation in aorta from spontaneously hypertensive rats. *Journal of Agricultural and Food Chemistry* 54, 2096-2102.

Sánchez-Salgado JC, Castillo-España P, Ibarra-Barajas M, Villalobos-Molina R, Estrada-Soto S. (2010). *Cochlospermum vitifolium* induces vasorelaxant and antihypertensive effects mainly by activation of NO/cGMP signaling pathway. *Journal of Ethnopharmacology* 130, 477-484.

Senthil S, Chandramohan G, Pugalendi KV. (2007). Isomers (oleanolic and ursolic acids) differ in their protective effect against isoproterenol-induced myocardial ischemia in rats. *International Journal of Cardiology* 119, 131-133.

Silva DF, Porto DL, Araújo IG, Dias KL, Cavalcante KV, Veras RC, Tavares JF, Correia NA, Guedes DN, Silva MS, Medeiros IA. (2009). Endothelium-derived nitric oxide is involved in the hypotensive and vasorelaxant effects induced by discretamine in rats. *Pharmazie* 64, 327–331.

Somova LO, Nadar A, Rammanan P, Shode FO. (2003). Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. *Phytomedicine* 10, 115-21.

Steinkamp-Fenske K, Bollinger L, Völler N, Xu H, Yao Y, Bauer R, Förstermann U, Li H. (2007a). Ursolic acid from the Chinese herb danshen (*Salvia miltiorrhiza* L.) upregulates eNOS and downregulates Nox4 expression in human endothelial cells. *Atherosclerosis* 195, 104-111.

Steinkamp-Fenske K, Bollinger L, Xu H, Yao Y, Horke S, Förstermann U, Li H. (2007b). Reciprocal regulation of endothelial nitric-oxide synthase and NADPH oxidase by betulinic acid in human endothelial cells. *The Journal of Pharmacology and Experimental Therapheutics* 322, 836-842.

Uydeş-Doğan BS, Takir S, Ozdemir O, Kolak U, Topçu G, Ulubelen A. (2005). The comparison of the relaxant effects of two methoxylated flavones in rat aortic rings. *Vascular Pharmacology* 43, 220-226.

World Health Organization. Prevention of Cardiovascular Disease: Pocket guidelines for assessment and management of cardiovascular risk. http://www.who.int/cardiovascular_diseases/guidelines/Pocket_GL_information/en/index.html. (Accessed 05.25.2011).

Zhang C, Wang XH, Zhong MF, Liu RH, Li HL, Zhang WD, Chen H. (2007) Mechanisms underlying vasorelaxant action of astragaloside IV in isolated rat aortic rings. *Clinical and Experimental Pharmacology & Physiology* 34, 387–92.

