Journal of Intercultural Ethnopharmacology
www.jicep.com
DOI: 10.5455/jice.20170706061922

Original Research

Exploratory studies of some Mexican medicinal plants: Cardiovascular effects in rats with and without hypertension

Gil Alfonso Magos-Guerrero, Jacinto Santiago-Mejía, Omar F. Carrasco*

Department of Pharmacology, School of Medicine, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico

Address for correspondence:
Omar F. Carrasco, Department of Pharmacology, School of Medicine, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico. E-mail: omar.carrasco.ortega@gmail.com

Received: April 17, 2017
Accepted: June 28, 2017
Published: July 12, 2017

ABSTRACT

Background: *Papaveraceae Argemone mexicana* L., *Burseraceae Bursera simaruba* (L.) *Sarg.*, *Acanthaceae Justicia spicigera Schltdl.* and *Selaginellaceae Selaginella lepidophylla* (Hook. & Grev.) Spring., have been used in Mexican traditional medicine to treat hypertension. The objective of this study was to further characterize the cardiovascular effects of the methanol extracts of such plants. **Methods:** The medicinal plants were collected and taxonomically identified; the methanol extract of each explored plant were administrated to conscious and unconscious male Wistar rats with and without glucose-induced hypertension. The blood pressure (BP) and heart rate (HR) were evaluated before and after the extract administration. Vascular reactivity experiments were conducted in rat aortic rings obtained from rats with and without sugar-induced hypertension, a model widely used to study such effects with cardiovascular agents. **Results:** After oral administration in normotensive conscious rats all tested extracts decreased the HR, such effect was only observed in hypertensive conscious rats after the administration of *B. simaruba*; only *A. mexicana* and *B. simaruba* decreased the BP after oral administration. All extracts administrated by intravenous injection diminished the mean arterial pressure. Dose-response curves to cumulative concentrations of all the extracts promote vascular relaxation in precontracted aortas from rats with and without sugar-induced hypertension. **Conclusions:** The present study indicated that *B. simaruba* is worthy of further investigation as a potential phytotherapeutic agent for treating hypertension.

KEY WORDS: *Argemone mexicana, Bursera simaruba*, hypertension, *Justicia spicigera, Selaginella lepidophylla,* traditional Mexican medicine

BACKGROUND

Hypertension is one of the most common conditions treated in primary care settings worldwide. It is an important preventable condition that leads to morbidity and mortality [1].

Fructose consumption, in the form of added sugars such as high fructose corn syrup or sucrose, has increased markedly in the past 30 years [2]. The excessive intake of fructose is one proposed cause of increased metabolic syndrome and obesity, and both conditions, in turn, are associated with the development of hypertension [3].

The current pharmacological options for hypertension treatment are wide and available [4]. With regard to the choice of antihypertensive agent, the 2013 European Society of Hypertension/European Society of Cardiology guidelines reconfirm that a diuretic, beta-blocker, calcium channel blocker, angiotensin II receptor blocker, and angiotensin-converting enzyme inhibitor are all suitable for use as monotherapy, and in some combinations with each other [5]; nevertheless, the quest for more safe and accessible pharmacological options is always present.

The traditional Mexican medicine uses empirically some plants as antihypertensive therapy; some of those plants are *Papaveraceae Argemone mexicana* L., *Burseraceae Bursera simaruba* (L.) Sarg., *Acanthaceae Justicia spicigera Schltdl.*, and *Selaginellaceae Selaginella lepidophylla* (Hook. and Grev.) Spring. [6]. Some scientific evidence could support this empiric use, *A. mexicana* promote capillary dilatation, proliferation, and increased capillary permeability leading to edema in humans over exposed to its oils [7,8] those effects could diminish blood pressure (BP); there are no publications that report vascular properties of *B. simaruba*, nevertheless physicochemical characterization detected the presence of proanthocyanidins [9], consumption of proanthocyanidins-rich foods, herbs, and beverages, is associated with an improvement in endothelial function through vascular endothelial nitric oxide synthase activation, that inductive fact could explain the vascular protecting effect of that plant [10], likewise structural analysis of *J. spicigera* detected eucalyptol as one of its main compounds [11], that essential oil promotes vascular smooth muscle relaxation [12] and could be responsible of the antihypertensive effect. *S. lepidophylla* promotes diuresis and also the major components isolated

from the plant include biflavonoids; those actions could validate its use as an antihypertensive drug [13].

The present study characterized the pharmacological influence of methanolic extracts of A. *mexicana*, *B. simaruba*, *J. spicigera*, and S. *lepidophylla* over BP and heart rate (HR) in rats with and without sugar-induced hypertension and correlated the pharmacological effects of those extracts *in vivo* versus the effects observed in rat aortic rings located at isolated organ chambers. This model allowed to recreate the most common cause of hypertension and to provide more evidence of the pharmacological properties of the studied extracts.

METHODS

Reagents

Noradrenaline (NA), acetylcholine (Ach), cloralose, urethane, and ascorbic acid were obtained from Sigma-Aldrich (St. Louis, MO USA). Sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), potassium monophosphate (KH_2PO_4), sodium carbonate acid ($NaHCO_3$), and glucose and methanol were obtained from J.T. Baker. Heparin was obtained from PiSA.

The Krebs–Henseleit solution consisted of the following composition: 127 mM NaCl; 4.7 mM KCl; 1.1 mM $MgSO_4$; 1.2 mM KH_2PO_4; 2.5 mM $CaCl_2$; 25 mM $NaHCO_3$; 11 mM glucose; and 0.02 mM ethylenediaminetetraacetic acid. The solution was kept at 37°°C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide; pH was 7.4. NA was dissolved in 0.1% ascorbic acid.

Preparation of the Methanolic Extracts

All plants were collected from already know places in Mexico from July to September 2016. Botanical samples were taxonomically identified at the Instituto Mexicano del Seguro Social by Master Abigail Aguilar Contreras, botanist in charge of the herbarium, the registry number assigned were 14,132 for A. *mexicana*, 14,136 for *B. simaruba*, 14,128 for S. *lepidophylla*, and 14,133 for *J. spicigera*.

Air-dried plant material (leaves) was powdered and stored in paper bags. The dried powdered material of the leaves (500 g) was extracted with methanol by percolation at room temperature; every 24 h the methanol was removed, and equivalent volume was added to the powder. This proceeding was repeated until the residue was <5% of the residue obtained in the first extraction. The solvent-free residues were mixed to constitute the full extract.

Animal Procedures

Male Wistar rats raised in the animal facilities of the School of Medicine, Universidad Nacional Autónoma de México, were used in all experiments. Rats were kept in animal rooms illuminated from 07:00 to 19:00 (12-h light/12-h dark cycles) and maintained at 21-23°C. The animals had free access to food pellets (Purina Chow, St. Louis, MO, USA) and tap water. Rats were brought daily to the laboratory for the experiments, which were conducted in accordance with the Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and were approved by the local Ethics Committee. Reference number 1348.

Each experimental group consisted of six rats; the animals were assigned to experimental groups by a chart of randomized numbers.

Some specimens were induced to develop hypertension by the addition of sugar in the drinking water for 5 months; this procedure began when the rats become 28 days old.

HR and BP in Conscious Rats

The rats were exposed on several occasions to the maneuvers used for indirect BP determinations: Placement in a restraining cylinder, application of external heat, repeated inflation, and deflation of a tail cuff and repeated installation of pulse transducer in the base of the tail. The signals were sent to computer software PRESION2. Only those animals eventually accepting these procedures without signs of undue distress were used for the experiments.

The trained rats were enclosed in a restraining cylinder and gently warmed with a lamp to insure adequate pulsation of the tail arteries. A pulse transducer (LE 5160/60) was placed at the base of the tail to detect arterial pulsations. All measurements were carried out in triplicate.

The HR and BP were previously assessed by the administration of 100 mg/kg of the extract and 0.5, 1, 2, 4, 6, 8, 24, and 48 h after the administration.

Data are expressed as values of mean ± standard error mean. The significance of pressure and rate changes at the different times after the extracts administration was evaluated by ANOVA.

HR and BP in Unconscious Rats

Male Wistar rats weighing between 200 and 300 g were anesthetized with chloralose, 50 mg/kg, and urethane, 750 mg/kg, both administered intraperitoneum. Cannulas were inserted into a femoral artery and vein for HR/BP recording and drug administration, respectively. Mean BP was recorded continuously with a transducer Statham P. 231D and the HR was recorded in a Grass 7P4 f polygraph system. The signal was recorded at PREFRE-EME software.

In a first series of experiments, hypotensive responses to increasing doses of the extracts (1, 3.1, 10, 31, and 100 mg/kg) were obtained in six rats without hypertension; since the extract were diluted in isotonic NaCl solution other group of rats without hypertension were tested to obtain a control group (0.1 mL/100 g of isotonic NaCl).

In a second experimental series, we reproduced the procedure described above in rats with glucose-induced hypertension. Responses in rats without hypertension and rats with hypertension were compared to corresponding controls by one-way ANOVA and Dunnett's test; $P < 5\%$ was considered as indicating statistical significance.

Rat Aorta Relaxation Experiments

The thoracic aorta was removed, and segments were obtained and suspended in organ chambers between two nickel–chromium wire hooks. One of the hooks was fastened to the bottom of the chamber and the other was attached to a Grass FT03 force transducer, which was connected in turn to computer TENSIN 41 software. The baths contained Krebs–Henseleit solution kept at 37°C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide; pH was 7.4. After a stabilization period of at least 60 min, during which the rings were stimulated several times with 100 nM NA, the integrity of the endothelium was assessed by verifying that the contracted preparations relaxed by at least 50% when challenged with 1 μM Ach. Endothelium was removed in some rings by rubbing intraluminally with a 20-gauge hypodermic needle; in these preparations, the absence of the endothelium was confirmed by a <10% relaxation on acetylcholine challenge. All experimental groups consisted of 7-9 rings.

The influence of increasing doses (1, 31, 100, 310, and 1000 μg/kg) of the extracts on endothelium-dependent and -independent relaxation was assessed in rings contracted with 100 nM noradrenaline.

Responses to individual concentrations of the extracts in the control and treated groups were compared using either an unpaired *t*-test or a one-way analysis of variance followed by Dunnett's *post hoc* test, with $P < 0.05$ considered statistically significant.

Statistical Analysis

Statistical analyses were performed using Origin 7.0® Software (Statistical Software Package for Windows, version 19). The data were expressed as the mean ± standard deviation, and differences are considered to be statistically significant at $P < 0.05$.

RESULTS

Conscious Rats

Table 1 shows initial values of HR and BP in normotensive and hypertensive rats.

HR and BP in the hypertensive group were statistically significant higher ($P < 0.05$) in comparison to the normotensive group that data corresponded to the described in the literature [14].

HR

A. mexicana and *J. spicigera*
The single oral administration of the extract reduced the HR in the normotensive rats [Figure 1], this effect was not observed in the hypertensive group.

B. simaruba
The single oral administration of 100 mg/kg *B. simaruba* decreased the HR in rats with normal BP and in rats with glucose-induced hypertension, the onset of this effect was observed immediately after administration and the maximal effect was observed roughly 8 h after the pharmacological intervention in both groups and was maintained after 50 h [Figure 2].

Table 1: Initial values of HR and BP in normotensive and hypertensive rats

Group	BP	BP	HR	HR
	Normotensive	Hypertensive	Normotensive	Hypertensive
Control	104±1	139±3	358±2	370±7
Argemone mexicana	114±2	138±2	329±4	354±5
Bursera simaruba	115±3	144±2	304±5	336±9
Selaginella lepidophylla	114±2	136±6	371±5	398±6
Justicia spicigera	107±1	136±1	327±8	376±3

Values expressed as means±standard error $n=6$. HR: Heart rate, BP: Blood pressure

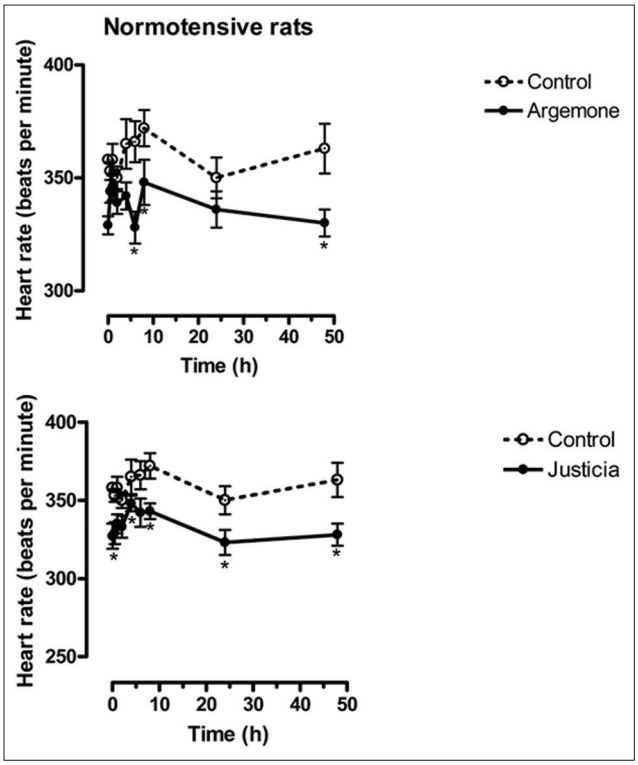

Figure 1: Influence of a single oral administration of *Argemone mexicana* or *Justicia spicigera* over heart rate in normotensive rats

S. lepidophilla

The single oral administration of 100 mg/kg *S. lepidophilla* slightly decreased the HR in rats with normal BP, in rats with induced hypertension the HR was increased [Figure 3].

BP

A. mexicana and B. simaruba

The single oral administration of the extracts diminished the BP in the hypertensive group of rats. No significant changes were observed in the normotensive group [Figure 4].

S. lepidophylla and J. spicigera

The single oral administration of the extracts did not modify the BP in the normotensive or the hypertensive group of rats.

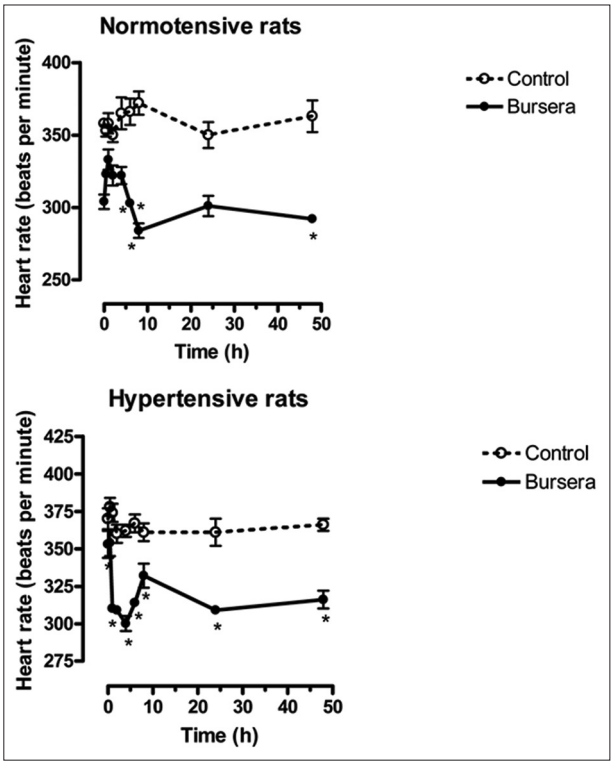

Figure 2: Influence of a single oral administration of *Bursera simaruba* over heart rate in normotensive and hypertensive rats

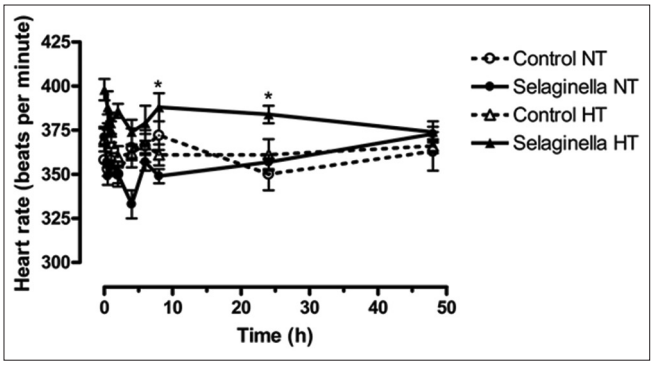

Figure 3: Influence of a single oral administration of *Selaginella lepidophilla* over heart rate in normotensive and hypertensive rats

Unconscious Rats

HR

The intravenous administration of *A. mexicana* increased the HR in the normotensive group and in the hypertensive group of rats, this effect was dose-dependent. The opposite effect was observed when *B. simaruba* and *S. lepidophylla* were administrated, both decreased the HR in the normotensive group and in the hypertensive group of rats this effect was dose-dependent [Figure 5]. *J. spicigera* administration did not modify the HR in the normotensive group or in the hypertensive group of rats.

Mean arterial pressure (MAP)

The intravenous administration of *A. mexicana*, *B. simaruba*, *S. lepidophylla*, and *J. spicigera* diminished the MAP in the normotensive group and in the hypertensive group of rats, this effect was dose-dependent [Figure 6a].

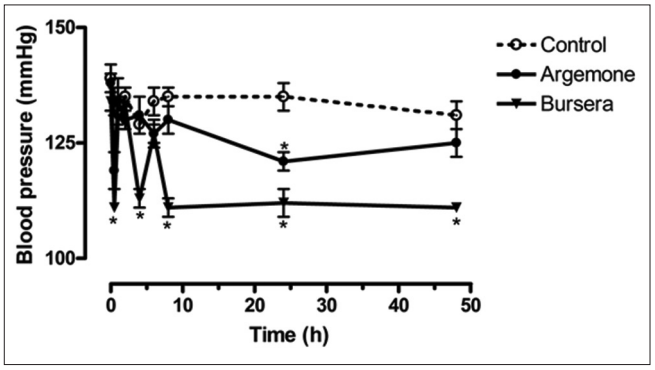

Figure 4: Influence of a single oral administration of *Argemone mexicana* or *Bursera simaruba* over blood pressure in hypertensive rats

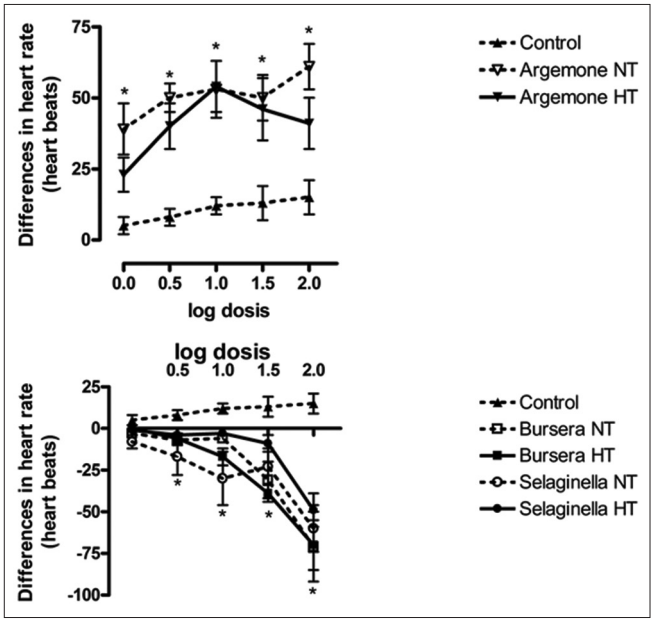

Figure 5: Influence of intravenous administration of *Argemone mexicana, Bursera simaruba* and *Selaginella lepidophilla* over heart rate in normotensive and hypertensive rats

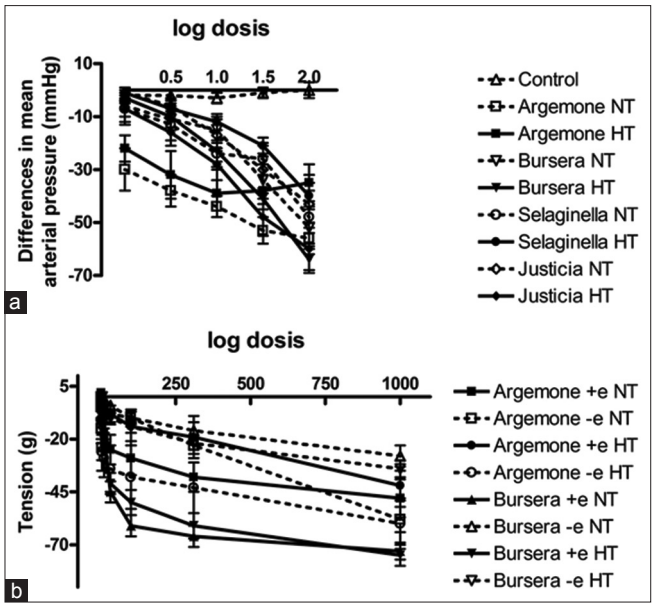

Figure 6: (a) Influence of intravenous administration of *Argemone mexicana, Bursera simaruba, Selaginella lepidophylla* and *Justicia spicigera* over mean arterial pressure in normotensive and hypertensive rats. (b) Aortic vascular reactivity due increasing concentrations of *A. mexicana* and *B. simaruba* in normotensive and hypertensive rats

Aortic rings

The increasing concentrations of A. *mexicana* and *B. simaruba* produced vascular relaxation in pre-contracted aortas from normotensive and hypertensive rats, such effects were concentration dependent in the vessels with endothelium and without endothelium [Figure 6b].

DISCUSSION

The explored substances are Mexican medicinal plants used for traditional treatment of hypertension. Some of them showed multiple and interesting vascular effects. *J. spicigera* and *S. lepidophylla* diminishes the HR in rats without sugar-induced hypertension, but they did not modify the HR in rats with hypertension or BP; these findings suggest that their therapeutic benefits could be modest. A. *mexicana* reduced the HR in rats without hypertension and showed no effect in the group with hypertension, despite that fact, reduced the BP in conscious and unconscious rats, and promoted vascular relaxation in rat aortic rings; all this data together could suggest further experiments with the extract for therapeutic purposes.

B. simaruba is one of the traditional plants with most scientific evidence in many fields, possess cytotoxicity, and antiviral properties against herpes simplex viruses (HSV-1 and HSV-2) [15], antifungal activity [16], antibacterial [17-19], anti-inflammatory [20-23], the present study describes some cardiovascular properties; was the only tested extract that reduced the HR in rats with and without hypertension, the only with negative chronotropic effect in all experimental groups, evoking the effects of the beta blockers [24-26], further experiments must test if this effect is observed or not in models of sympathetic denervation [27] or in the presence of selective adrenergic antagonist to elucidate the pharmacodynamic mechanism of action. The single oral administration of the extract decreased the BP for more than 40 h; antihypertensive drugs action should last at least 24 h to enhance adherence [28,29], postulating it as a novel long-acting antihypertensive drug. The extract promoted vascular relaxation in aortic rings from rats with and without hypertension, this effect was more evident in the rings with the endothelial layer preserved, the hypertension model used in this work, promote some special disturbances in the vascular homeostasis, one of them is the endothelial dysfunction [30,31], the endothelial function impairment could diminish the release of vasodilator mediators as nitric oxide (NO) or promote the liberation of vasoconstrictor ones as endothelin-1 (ET-1) known also for mitogenic effects, and for deteriorate the process of hypertension and atherosclerosis by aggravating hyperplasia and migration in vascular smooth muscle cells [32,33], the effects observed suggests that the extract promote the NO availability in the vascular compartment, or possibly, it could limit the release of ET-1 from endothelial cells, restoring the balance between the vasodilator and the vasoconstrictor mediators in the vascular compartment. Further exploration could be conducted in the presence of N-Nitro-L-arginine methyl ester hydrochloride, an analog of arginine that inhibits NO production and observe if this effect is mediated by nitric oxide, or in the presence of indomethacin, an unspecific cyclooxygenase inhibitor to observe if the effect is mediated by prostaglandins.

CONCLUSION

B. simaruba possess an interesting cardiovascular profile characterized by negative chronotropic effects and long-term hypotension induced by a single oral administration of the extract and vasodilator properties that could be endothelium protectant. A. *mexicana* diminishes the BP in conscious and unconscious rats; nevertheless, it increased the HR in rats with hypertension action that discourage further explorations. *S. lepidophylla* and *J. spicigera* did not decrease the BP.

The present study indicated that *B. simaruba* is worthy of further investigation as a potential phytotherapeutic agent for treating hypertension.

REFERENCES

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, *et al.* Heart disease and stroke statistics–2015 update: A report from the American Heart Association. Circulation 2015;131:e29-322.
2. Madero M, Lozada LG, Johnson RJ. Fructose likely does have a role in hypertension. Hypertension 2012;59:e54.
3. Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH, *et al.* Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr 2007;86:899-906.
4. Farooq U, Ray SG. 2014 guideline for the management of high blood pressure (eighth joint national committee): Take-home messages. Med Clin North Am 2015;99:733-8.
5. Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M, *et al.* 2013 ESH/ESC guidelines for the management of arterial hypertension: The task force for the management of arterial hypertension of the European Society of Hypertension (ESH)

and of the European Society of Cardiology (ESC). J Hypertens 2013;31:1281-357.
6. Martínez M. Las Plantas Medicinales de México. 6th ed. México: Ediciones Botas; 1990.
7. Sharma BD, Malhotra S, Bhatia V, Rathee M. Epidemic dropsy in India. Postgrad Med J 1999;75:657-61.
8. Sachdev MS, Sood NN, Mohan M, Sachdev HP, Gupta SK. Optic disc vasculitis in epidemic dropsy. Jpn J Ophthalmol 1987;31:467-74.
9. Maldini M, Montoro P, Piacente S, Pizza C. ESI-MS, ESI-MS/MS fingerprint and LC-ESI-MS analysis of proathocyanidins from *Bursera simaruba* Sarg bark. Nat Prod Commun 2009;4:1671-4.
10. Hügel HM, Jackson N, May B, Zhang AL, Xue CC. Polyphenol protection and treatment of hypertension. Phytomedicine 2016;23:220-31.
11. Baqueiro-Peña I, Guerrero-Beltrán JÁ. Physicochemical and antioxidant characterization of *Justicia spicigera*. Food Chem 2017;218:305-12.
12. Soares MC, Damiani CE, Moreira CM, Stefanon I, Vassallo DV. Eucalyptol, an essential oil, reduces contractile activity in rat cardiac muscle. Braz J Med Biol Res 2005;38:453-61.
13. Aguilar MI, Benítez WV, Colín A, Bye R, Ríos-Gómez R, Calzada F. Evaluation of the diuretic activity in two Mexican medicinal species: *Selaginella nothohybrida* and *Selaginella lepidophylla* and its effects with ciclooxigenases inhibitors. J Ethnopharmacol 2015;163:167-72.
14. Kiriyama A, Honbo A, Nishimura A, Shibata N, Iga K. Pharmacokinetic-pharmacodynamic analyses of antihypertensive drugs, nifedipine and propranolol, in spontaneously hypertensive rats to investigate characteristics of effect and side effects. Regul Toxicol Pharmacol 2016;76:21-9.
15. Álvarez ÁL, Habtemariam S, Parra F. Inhibitory effects of lupene-derived pentacyclic triterpenoids from *Bursera simaruba* on HSV-1 and HSV-2 *in vitro* replication. Nat Prod Res 2015;29:2322-7.
16. Biabiany M, Roumy V, Hennebelle T, François N, Sendid B, Pottier M, *et al.* Antifungal activity of 10 Guadeloupean plants. Phytother Res 2013;27:1640-5.
17. Rosas-Piñón Y, Mejía A, Díaz-Ruiz G, Aguilar MI, Sánchez-Nieto S, Rivero-Cruz JF. Ethnobotanical survey and antibacterial activity of plants used in the Altiplane region of Mexico for the treatment of oral cavity infections. J Ethnopharmacol 2012;141:860-5.
18. Junor GO, Porter RB, Facey PC, Yee TH. Investigation of essential oil extracts from four native Jamaican species of *Bursera* for antibacterial activity. West Indian Med J 2007;56:22-5.
19. Yasunaka K, Abe F, Nagayama A, Okabe H, Lozada-Pérez L, López-Villafranco E, *et al.* Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumarins and xanthones. J Ethnopharmacol 2005;97:293-9.
20. Carretero ME, López-Pérez JL, Abad MJ, Bermejo P, Tillet S, Israel A, *et al.* Preliminary study of the anti-inflammatory activity of hexane extract and fractions from *Bursera simaruba* (Linneo) Sarg. (*Burseraceae*) leaves. J Ethnopharmacol 2008;116:11-5.
21. Zúñiga B, Guevara-Fefer P, Herrera J, Contreras JL, Velasco L, Pérez FJ, *et al.* Chemical composition and anti-inflammatory activity of the volatile fractions from the bark of eight Mexican *Bursera* species. Planta Med 2005;71:825-8.
22. Noguera B, Díaz E, García MV, Feliciano AS, López-Perez JL, Israel A. Anti-inflammatory activity of leaf extract and fractions of *Bursera simaruba* (L.) Sarg (*Burseraceae*). J Ethnopharmacol 2004;92:129-33.
23. Sosa S, Balick MJ, Arvigo R, Esposito RG, Pizza C, Altinier G, *et al.* Screening of the topical anti-inflammatory activity of some Central American plants. J Ethnopharmacol 2002;81:211-5.
24. Ikeshita K, Nishikawa K, Toriyama S, Yamashita T, Tani Y, Yamada T, *et al.* Landiolol has a less potent negative inotropic effect than esmolol in isolated rabbit hearts. J Anesth 2008;22:361-6.
25. Pádua-Filho WC, Brasil DP, Neves HJ, Gomes OM, Bocchi EA. Effects of metoprolol and amiodarone combination on heart rate, myocardial contractility and coronary flow: Study in isolated perfused rat hearts. Exp Clin Cardiol 2004;9:133-7.
26. Yamakawa H, Takeuchi M, Takaoka H, Hata K, Mori M, Yokoyama M. Negative chronotropic effect of beta-blockade therapy reduces myocardial oxygen expenditure for non mechanical work. Circulation 1996;94:340-5.
27. Dos Reis DG, Fortaleza EA, Tavares RF, Corrêa FM. Role of the autonomic nervous system and baroreflex in stress-evoked cardiovascular responses in rats. Stress 2014;17:362-72.
28. Bendersky M, Juncos L, Waisman GD, Piskorz D, Lopez-Santi R, Montaña O, *et al.* Abpm and duration of the antihypertensive effect: A study with a new formulation of sustained release losartan (CRONOS). Rev Fac Cien Med Univ Nac Cordoba 2012;69:213-8.
29. Graney WF. Clinical experience with a once-daily, extended-release formulation of diltiazem in the treatment of hypertension. Am J Med 1992;93:56S-64.
30. Chou CL, Pang CY, Lee TJ, Fang TC. Beneficial effects of calcitriol on hypertension, glucose intolerance, impairment of endothelium-dependent vascular relaxation, and visceral adiposity in fructose-fed hypertensive rats. PLoS One 2015;10:e0119843.
31. Wang LP, Jiang Y, Yang H, Peng C, Zhang C, Tao X, *et al.* Combination therapy of nifedipine and sulphonylureas exhibits a mutual antagonistic effect on the endothelial cell dysfunction induced by hyperglycemia linked to vascular disease. Cell Physiol Biochem 2016;38:2337-47.
32. Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, *et al.* Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood 1998;91:3527-61.
33. Lin YJ, Juan CC, Kwok CF, Hsu YP, Shih KC, Chen CC, *et al.* Endothelin-1 exacerbates development of hypertension and atherosclerosis in modest insulin resistant syndrome. Biochem Biophys Res Commun 2015;460:497-503.

Source of Support: Universidad NacionalAutónoma de México give all sources of funding for the research, Conflict of Interest: None declared.