ANTIHYPERTENSIVE AND VASORELAXANT EFFECTS OF THE AQUEOUS EXTRACT OF C*asimiroa edulis*

Vázquez-Cruz B, Vázquez-Muñoz M, Navarrete-Bastida R, Trujillo-González I.D, De Haro R, Segura-Cobos D. Muñoz-López JL

Laboratorio de Farmacología. UIICSE, FES-Iztacala. UNAM. Los Reyes Iztacala. Tlalnepantla, Estado de México 54090, MEXICO. E-mail: bvcruz@servidor.unam.mx

Summary

The aqueous extract of *Casimiroa edulis* leaves was examined on three models of experimental hypertension and on the vascular reactivity in the isolated perfused kidney. The administration of the aqueous extract (100 mg/Kg body weight) diminished the maximal increase of systolic blood pressure in angiotensin II-treated rats from 35.5 ± 4.6 mmHg to 10.4 ± 6.3 mmHg. Furthermore, the blood pressure of rats made hypertensive by aortic coarctation and by N^G-nitro-L-arginine methyl ester (L-NAME) administration decreased. The vascular response to angiotensin II in the renal circulation decreased in rats with aortic coarctation treated with the aqueous extract of *Casimiroa edulis* leaves; similarly, the vascular response to phenylephrine also decreased in kidneys from rats made hypertensive by L-NAME and treated with the aqueous extract of *Casimiroa edulis* leaves. These data suggest that the aqueous extract of *Casimiroa edulis* leaves has antihypertensive effect that justifies its use as an antihypertensive agent in traditional medicine.

Key words: *Casimiroa edulis*, Hypertension, Antihypertensive effect, Aortic coarctation, N^G-nitro-L-arginine methyl ester (L-NAME).

Introduction

Casimiroa edulis la Llave & Lex. (Fam. Rutaceae), is a native tree that grows in the temperate zones of Mexico and Central America. It is commonly known as zapote blanco, and its name in Nahuatl is cochitzapotl (1). Its leaves and seeds are used in traditional medicine by their sedative and sleep-inducing effects and to treat hypertension (2, 3). Several studies have demonstrated that the hydroalcoholic extract of *C. edulis* possesses sedative and antidepressant properties in rodents, as well as anticonvulsive activity in models of experimental epilepsy in rats (4, 5).

On the other hand, studies carried out by Magos and Vidrio (6) showed that the aqueous and alcoholic extracts of *C. edulis* seeds cause hypotension in normotensive animals and vasorelaxation in isolated organs (7). bioassay-directed fractionation of the methanolic extract of *C. edulis* seeds led to the isolation of seven components with cardiovascular activity (8). Because people usually do not use the seeds but the leaves or the stems to treat their health problems, we were interested in studying whether *C. edulis* leaves also have antihypertensive effects besides the cardiovascular effects already known, therefore, the purpose of this study was to evaluate the possible antihypertensive effect of the aqueous extract of *C. edulis* leaves by using three models of experimental hypertension, as well as on the vascular reactivity of the isolated perfused rat kidney.

Methods

Preparation of the plant extract

C. edulis leaves were collected in Tlaxcala, Mexico between March and April 2008, and identified by biologist Edith Lopez Villa-Franco. Voucher specimen (28906) is kept at the Herbarium Izta of the Iztacala Faculty, a branch faculty of the National Autonomous University of Mexico (UNAM). The aqueous extract was prepared with 10g pulverized leaves treated with 500 ml distilled water for 60 min at 80 ^{O}C and then centrifuged at 100 x g for 10 min to remove particulate matter. The supernatant was vacuum dried in a Model 75050 Labcon freeze dryer for storage until the pharmacological assays were carried out. Each 10g of powdered dried leaves yielded 320 mg of lyophilized powder. The solid aqueous extract thus obtained was dissolved in isotonic NaCl solution (0.9%) just before administration.

Animals

Adult male Wistar rats weighing between 300 to 350g were used throughout the experiments. The animals were kept in the animal facility of the Iztacala Faculty and were housed at 24 ± 0.5^{o}C with a 12 h:12 h light/dark cycle and free access to rat chow and water. All animal procedures were conducted according to the National Institute of Health Guide for the Care of Laboratory Animals.

Animal preparation for blood pressure measurement and dose-response curves to *C. edulis* and angiotensin II in normotensive rats.

Rats were anesthetized with sodium pentobarbital (45 mg/kg body weight by i.p. injection). The trachea was cannulated and the animals were artificially ventilated using a Palmer respirator (rate 48 strokes /min., stroke volume 1.5 mL/100 mg body weight). The femoral vein and carotid artery were isolated and cannulated for the administration of plant extract and angiotensin II and blood pressure measured, respectively. The systolic blood pressure (SBP) was measured with a pressure transducer (Narco Bio-System model P1000B). The signal from the transducer was recorded on a physiographer (model DMP-4B). The SBP was allowed to stabilize for at least 20 min before the administration of a test substance. We carried out dose-response curves to the aqueous extract of *C. edulis* leaves (10-100 mg/kg) and then, when the SBP returned to basal values, dose-response curves to angiotensin II (25-250 ng/kg body weight, Sigma Chemical Co., St. Louis, MO, USA), with or without the aqueous extract of *C. edulis* (100 mg/kg body weight) were also conducted.

Hypertension induction by aortic coarctation

Animals were randomly allocated into six groups of six rats each. Surgical procedures were performed with diethyl ether as anesthetic. For this, the aorta was exposed through the abdominal cavity and partially ligated at a point between the right and left renal artery, reproducibility was achieved with a 19-gauge needle (9). The control group was surgically intervened, but the aorta was not ligated (sham surgery). The next day of the aortic coarctation, the experimental groups received the aqueous extract of *C. edulis* by intragastric route at a dose of 100 mg/kg body weight (group 1), 300 mg/kg body weight (group 2) and 600 mg/kg body weight (group 3). These doses were chosen because they are related to the doses employed in the hypertension treatment by Mexican traditional medicine. Enalapril (5 mg/kg body weight) was used as standard drug (group 4). The control group received vehicle in the same volume of saline solution (0.1 mL/100 g) and by the same route (group 5). After 28 days of treatment, the animals were anesthetized with sodium pentobarbital and their SBP was recorded through the carotid artery as previously described. Animals were considered hypertensive only when a difference ≥ 40mm Hg between aortic coarctation and sham-operated animals was found. Then a laparotomy was performed and the right kidney was removed to evaluate the vascular reactivity in the isolated perfused kidney.

L-NAME-induced hypertension

Animals were randomly allocated into six groups of six animals each and for ten days. Group 1 (control) only received the vehicle, group 2 received L-NAME (N^G-nitro-L-arginine methylester; 70 mg/kg body weight), while groups 3 to 5 received L-NAME plus the aqueous extract of *C. edulis* leaves (100, 300 and 600 mg/kg, respectively), and group 6 was given enalapril (5 mg/kg body weight). All drugs were administered in drinking water and the actual doses of each group were calculated from the daily water intake. After this, the rats were anesthetized with sodium pentobarbital and their SBP was recorded through the carotid artery as previously described, then a laparotomy was performed and the right kidney was removed to evaluate the vascular reactivity in the isolated perfused kidney experimental model.

Vascular reactivity in the isolated perfused kidney of the rat

The groups of rats with aortic coarctation and /or treated with L-NAME were anesthetized with sodium pentobarbital (45 mg/kg i.p). The right kidney was exposed and the mesenteric and right renal arteries were trimmed of surrounding connective tissue. Ties were loosely placed around these vessels and the vena cava. The right renal artery was then cannulated with a 19-gauge needle via the mesenteric artery to avoid total interruption of blood flow, and the vena cava was occluded and cut to provide an outlet for the perfusate. The right ureter was also cut and the animals were killed by an intracardiac injection of 10 mg sodium pentobarbital. The kidney was removed, suspended in a water-jacketed bath at 37^OC, and perfused at a constant flow by using a Watson-Marlow peristaltic pump (model 502S: New Brunswick Scientific, Edison. NJ. U.S.A) with Krebs solution at 37 ^OC and gassed with O_2/CO_2 (95%:5%). The Krebs solution used had the following mM composition: 118 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 4.2 $MgSO_4$, 25 $NaHCO_3$, and 11.5 of glucose at pH 7.4. The flow was adjusted to obtain a basal perfusion pressure of 80-90 mm Hg. Mean flow rate of the perfusate was 9.0 ± 0.4 ml/min. The perfusion pressure was measured with a Harvard Transducer model 377 (Harvard apparatus Company Inc., South Natick, MA, U.S.A) and recorded on a Sybron recorder (Baxter Diagnostics Corp., California).

Because the flow was maintained at a constant rate, a change in the perfusion pressure represented a change in the resistance of the renal vasculature. An increase in the perfusion pressure indicated vasoconstriction. The peristaltic pump produced a pulsatile pressure with maximal and minimal values, which were not electronically averaged. Changes in perfused pressure brought about by vasoconstrictors were calculated by taking the mean value of trace at the maximal perfusion pressure value after injection of such chemical. Data are expressed as changes (Δ) of the perfusion pressure in mm Hg. After a 20 to 30 min equilibration period, various doses of angiotensin II (10 to 80 ng) were randomly administered as a bolus into the perfusate line, proximal to the kidney from sham-operated, aortic coarctated rats treated with or without the aqueous extract of *C. edulis* and enalapril. Each dose of angiotensin II was administered once the perfusion pressure had returned to basal values. Two experiments were performed on the same day, one for the kidney from sham-operated rats and another for rats with aortic coarctation treated with or without *C. edulis*, alternating the order during the following days.
Vascular reactivity to phenylephrine was also measured by the administration of various doses (0.2 to 1.6 μg) into the right kidney obtained from L-NAME-treated rats. The procedure was similar to that used with angiotensin II.

Statistical analysis
Data were expressed as mean ± standard error (SEM). Statistical differences between means were evaluated using the Student´s t-test for unpaired and paired observations, and using one-way ANOVA followed by the Newman-Keuls post-test, for multiple comparisons between more than two treatment groups. Differences were considered statistically significant if $P < 0.05$.

Results

The systolic blood pressure (SBP) in anesthetized rats was 110 ± 3.93 mmHg, only the 10 mg/kg body weight dose of the aqueous extract of *C. edulis* had a transient hypotensive effect (-14.72 ± 2.04 mmHg) that lasted less than 1 minute, while injections of higher doses did not significantly modify the SBP (Fig.1).

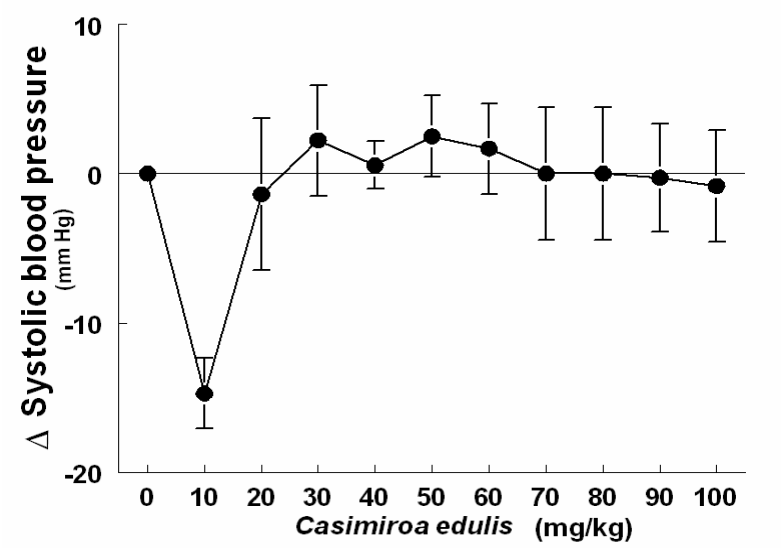

Figure 1: Effect of the aqueous extract of *C. edulis* leaves (10-100 mg/kg body weight) on the SBP in normotensive rats. Each point of the curve represents the mean (n=6) ± SEM.

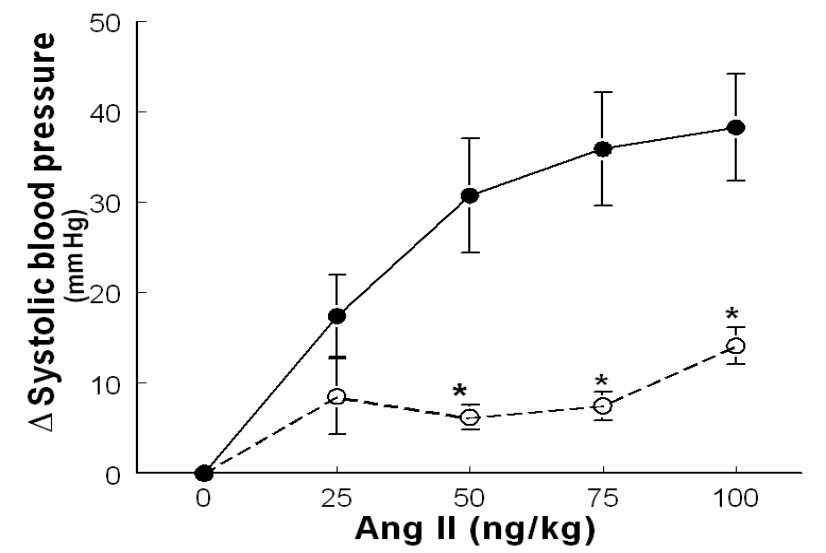

Figure 2: Effect of angiotensin II on SBP response in normotensive rats, with (○) or without (●) the previous administration of the aqueous extract of *C. edulis* (100 mg/kg body weight). Angiotensin II was administered in doses of 10-100 ng/kg body weight. (n=6) ± SEM, *p<0.05 was considered statistically significant.

The administration of angiotensin II increased the SBP of rats in a dose-dependent manner. The maximal increase of the SBP with 100 ng/kg body weight of angiotensin II was 38.5 ± 6.3 mmHg, whereas in the presence of the aqueous extract of *C. edulis* (100 mg/kg) with the same angiotensin II concentration, the SBP increased 10.4 ± 2.2 mmHg (Fig. 2).

The SBP in normotensive rats was 101.2 ± 12 mmHg, whereas in aortic coarctatated hypertensive rats the SBP was 163.8 ± 6.2 mmHg. The treatment of hypertensive rats with 100, 300 and 600 mg/kg body weight of the aqueous extract of *C. edulis* leaves decreased SBP to 136.33 ± 7.5, 125.8 ± 8.5 and 110.3 ± 15 mmHg, respectively. Inhibition of the angiotensin-converting enzyme with enalapril (5 mg/kg) restored the SBP values close to normotensive values of 119.0 ± 5.4 mmHg (Fig. 3).

The administration of Ang II increased the renal perfusion pressure of isolated perfused kidneys from sham-operated and aortic coarctated rats in a dose-dependent manner. However, the increase in perfusion pressure was higher in the kidneys from rats with aortic coarctation. The maximal increase of renal perfusion pressure with 80 ng Ang II was 78.0 ± 8.8 mmHg vs 32.8 ± 5.2 mm Hg of the normotensive rats (data not showed). When dose-response curves to angiotensin II were performed in the kidneys from aortic coarctated rats that received treatment with the aqueous extract of *C. edulis* at a dose of 300 and 600 mg/kg, the maximal responses were reduced to 57.4 ± 8.4 mmHg and 33.8 ± 8.0 mmHg, respectively (Fig 4).

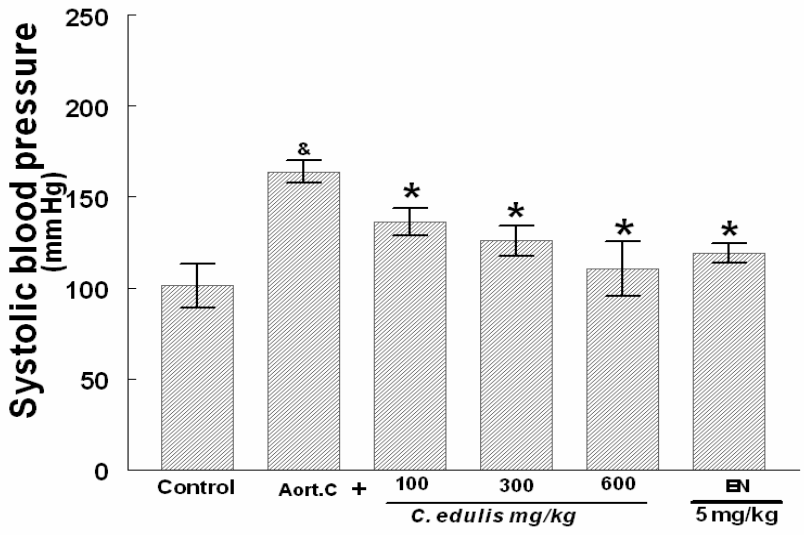

Figure 3: Effect of oral administration of the aqueous extract of *C. edulis* leaves (100, 300 and 600 mg/kg body weight) and enalapril on blood pressure in anesthetized aortic coarctated rats previously treated with *C. edulis*. Each bar represents the mean (n=6) ± SEM,*$p<0.05$ was considered statistically significant.

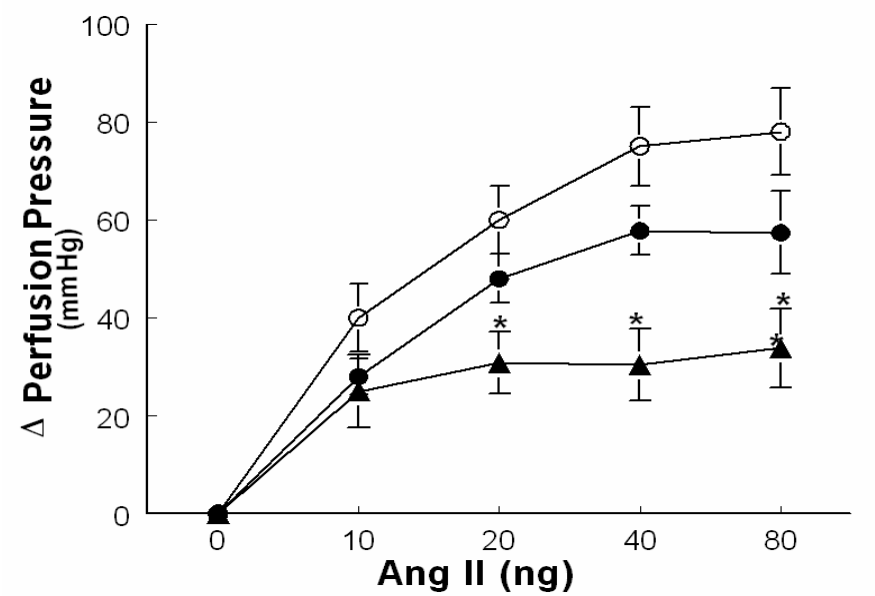

Figure 4: Effect of the aqueous extract of *C. edulis* leaves on angiotensin II (Ang II)-induced renal vasoconstriction. Isolated perfused kidneys from aortic coarctated rats were stimulated with a bolus injection of 10-80 ng of Ang II. Control group (○) and groups treated with 285 (●) and 570 (▲) mg/Kg body weight *C. edulis* for 28 days, respectively. (n=6) ± SEM, *p<0.05 was considered statistically significant.

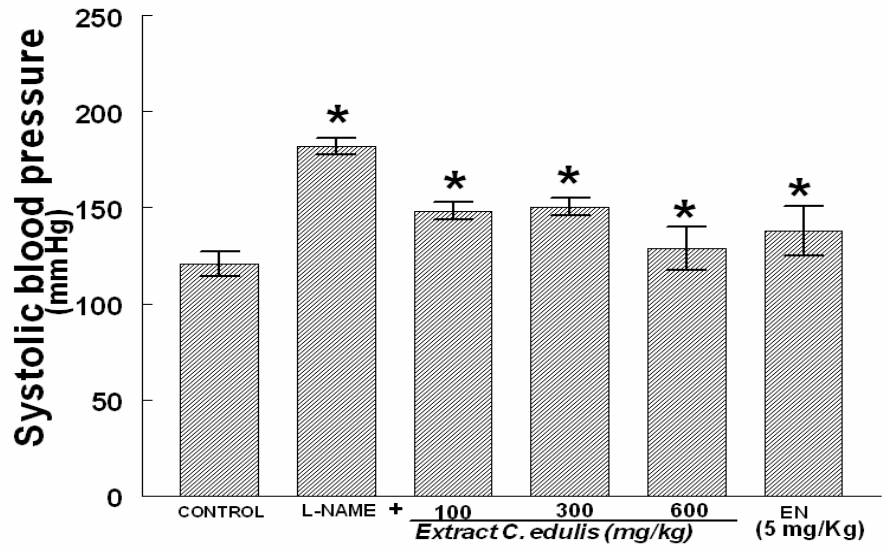

Figure 5: Effect of the oral administration of the aqueous extract of *C. edulis* leaves (100, 300 and 600 mg/kg body weight) on blood pressure in anesthetized L-NAME-treated rats. (n=6) ± SEM. *p<0.05 was considered statistically significant.

The administration of L-NAME for ten days increased blood pressure levels. Thus blood pressure in normotensive rats (control group) was 120.5 ± 3.9 mmHg, whereas in hypertensive rats blood pressure was 180.3 ± 4.5 mmHg. The treatment of such animals with *C. edulis* at doses of 100, 300 and 600 mg/kg body weight decreased blood pressure levels to 148.32 ± 4.4 mmHg, 150.21± 4.6 mmHg and 128.67 ± 11.2 mmHg, respectively. The treatment with enalapril (5 mg/kg) decreased the blood pressure level to 137.8 ± 12.8 mmHg (Fig 5).

When dose-response curves to phenylephrine were performed in isolated perfused kidneys obtained from rats made hypertensive with L-NAME, the maximal increase of renal perfusion pressure with 1.6 µg phenylephrine was 126 ± 1.7 mmHg. When dose-response curves were performed in the kidneys from such rats, the previous treatment with *C. edulis* at a dose of 100, 300 and 600 mg/kg body weight showed the maximal responses to be reduced to 89.0 ± 7.9, 60.0 ± 6.7, and 22.4 ± 11.6 mmHg, respectively (Fig 6).

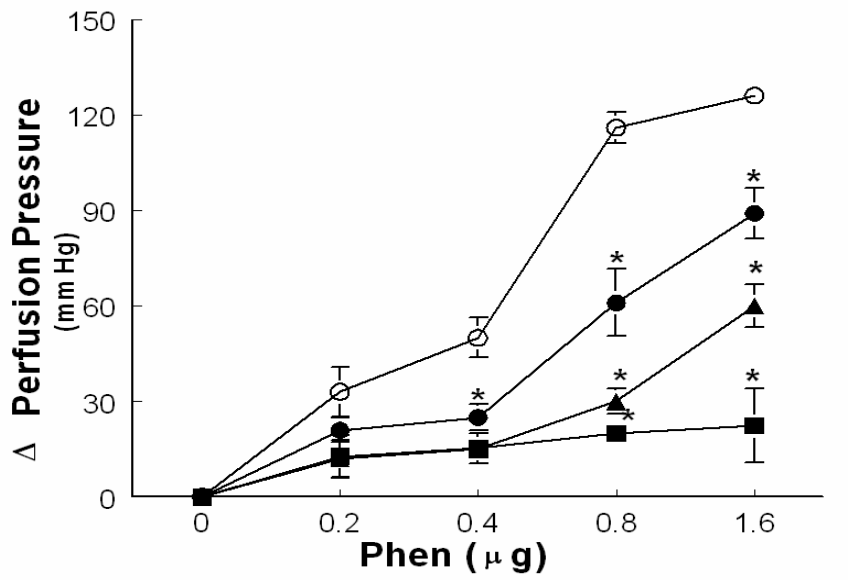

Figure 6: Effect of the aqueous extract of *C. edulis* leaves on phenylephrine (Phen)-induced renal vasoconstriction. Isolated perfused kidneys from rats treated with L-NAME for two weeks were stimulated with a bolus injection of 0.2-1.6 µg of Phen (control group (○)) and treated with *C. edulis* for two weeks whit doses of 300 mg/kg body weight (●) and 600 mg/Kg body weight (▲), respectively. (n=6) ± SEM.*$p<0.05$ was considered statistically significant.

Discussion

In this study we show that *C. edulis* produces a decrease of SBP in the experimental models of hypertension studied. The aqueous extract of *C. edulis* leaves had just a slight effect on the normal blood pressure similar to the aqueous and the methanolic extract of *C. edulis* seeds as was observed by Magos y Vidrio (6). This transitory effect could be attributed to the content of histamine-derived compounds like monomethylhistamine and dimethylhistamine, known to be present in this plant (7, 8). Angiotensin II plays a key role in the regulation of cardiovascular homeostasis affecting both blood pressure and fluid volume and is one of the most important etiological factors of hypertension (10). Angiotensin II administration in growing doses to normotensive rats was carried out to produce one model of acute hypertension. When the aqueous extract of *C. edulis* was administered at a dose of 100 mg/Kg, a decrease of the maximal effect of angiotensin II was observed, suggesting that the extract has chemicals able to antagonize the hypertensive effect of angiotensin II. The displacement of the dose-response curve of angiotensin II suggests a non-competitive antagonism or functional antagonism by stimulating the release of vasorelaxant compounds such as nitric oxide (11).

To study the antihypertensive effect of the aqueous extract of *C. edulis,* the aortic coarctation hypertension model was used, in which we observed that after 28 days of coarctation the rats showed hypertension as a consequence of reduced renal perfusion and subsequent activation of the renin-angiotensin system (12). The increase in blood pressure was lower in the aortic coarctated rats treated with the aqueous extract of *C. edulis* than in the aortic coarctated rats treated with saline solution. In a similar way, the angiotensin converting enzyme inhibitor enalapril also decreased blood pressure, but the aqueous extract of *C. edulis* at a dose of 600 mg/Kg body weight was more effective than 5 mg/Kg body weight enalapril. When the renal vascular reactivity was studied, we showed that the reactivity to angiotensin II of the renal vasculature was higher in hypertensive than in normotensive rats. These results are in agreement to data of other authors who attributed these differences to an increase in the metabolism of vasoconstrictor prostaglandins, contributing with the development of hypertension (12). On the other hand, the effect of angiotensin II on the renal perfusion pressure was antagonized in treated rats with the aqueous extract of *C. edulis* in a dose-dependent manner. These results suggest that *C. edulis* leaves contain chemicals able to decrease blood pressure in hypertensive rats and the vascular renal reactivity by altering the vasoconstrictor functions of the renin-angiotensin-aldosterone system. Indeed, several researchers have clearly shown that the acute and chronic inhibition of NO production induced by the oral administration of NO synthase inhibitors such as N^G-nitro-L-arginine methyl ester (L-NAME) causes marked rises in systemic blood pressure (13), confirming the main role of NO in maintaining the vascular smooth muscle tone and in regulating blood pressure (11). There is considerable evidence suggesting that the mechanisms responsible for the arterial hypertension produced after NO synthesis inhibition involves the overactivity of the renin-angiotensin system (14). In this study we show that the administration of L-NAME for ten days increased the SBP, and that the coadministration of the aqueous extract of *C. edulis* leaves diminished such increase, compared to the group of L-NAME-treated rats which only received vehicle. The angiotensin-converting enzyme inhibitor enalapril also decreased blood pressure, which confirms the participation of angiotensin II in this hypertension model (15).

The experiments of vascular renal reactivity carried out with kidneys from rats treated with L-NAME plus the aqueous extract of *C. edulis* showed that the responses to different doses of phenylephrine decreased the perfusion pressure in a dose-dependent manner. Recent research has indicated that several plant products exert their vascular effects by modifying the NO production (16-17). Our results show that the aqueous extract of *C. edulis* leaves decreases the SBP in hypertensive rats treated with L-NAME, which could be accomplished through compounds able to increase the NO release from endothelial cells, overcoming the inhibitory action of L-NAME over NOS. This seems plausible, because it has been shown in studies carried out on the mesenteric arterial bed in rats that the aqueous extract *C. edulis* seeds can induce its relaxation by stimulating the production of endothelial nitric oxide (18), although it could also be possible that the aqueous extract of *C. edulis* contains substances able to antagonize the renin-angiotensin aldosterone system.

In conclusion, this study provides pharmacological support that justifies the use of *Casimiroa edulis* in ethnomedical practices as antihypertensive agent. Further studies are in progress to identify the active constituents from extracts of this plant to allow us to understand its effects better.

Acknowledgements

This study was supported by a grant from the Facultad de Estudios Superiores Iztacala (PAPCA 2003) of the Universidad Nacional Autónoma de México. We are grateful to biologist Edith Lopez Villa-Franco by authentication of the plant.

References

1.- Martínez M. Las Casimiroas de México y Centroamérica. Anales del Instituto de Biología, México 1951; 22: 25-81

2.- Lozoya X. Enríquez R. El zapote blanco: investigación sobre una planta medicinal Mexicana. Consejo Nacional de Ciencia y Tecnología 1981. México.

3.- Mora S, Diaz-Velez G, Lungenstrass H, et al. Central nervous system activity of the hydroalcoholic extract of *Casimiroa edulis* in rats and mice. Journal of Ethnopharmacology. 2005;97: 191-197

4.- Navarro-Ruiz A, Bastidas-Ramirez B.E, García-Estrada J, et al. Anticonvulsivant activity *of Casimiroa edulis* in comparison to phenytoin and phenobarbital. Journal of Ethnopharmacology. 1995; 45: 199-206.

5.- Garzón-De la Mora P, García-López PM, García-Estrada J, et al. *Casimiroa edulis* seed extracts show anticonvulsivant properties in rats. Journal of Ethnopharmacology. 1999; 64: 275-282

6.- Magos J A, Vidrio H. Pharmacology of *Casimiroa edulis*; part 1. Blood pressure and Heart rate effects in the anesthetized rat. Planta Medica. 1991; 57: 20-24.

7.- Magos JA, Vidrio H, Enriquez R. Pharmacology of *Casimiroa edulis* III. Relaxant and contractile effects in rat aortic rings. Journal of Pharmacology. 1995; 47: 1-8.
8.- Magos J A, Vidrio H, Reynolds WF, et AL, Pharmacology of *Casimiroa eduli.* IV. Hypotensive effects of compounds isolated from methanolic extracts in rat and pigs. Journal of Ethnopharmacology. 1999; 64: 35-44
9.- Lin Lang, Nasjletti A. Role of prostaglandins in renin-dependent and renin-independent hypertension. Hypertension. 1991; 17: 517-525
10.- Sealy JE, Laragh JH. The renin- angiotensin-aldosterone system for normal regulation of blood pressure and sodium and potassium homeostasis. In: Laragh, J.H., Brenner, B.M. (Eds), Hypertension: Pathophysiology, Diagnosis and Management, 2nd ed. Raven Press, New York, NY. 1995: 1763-1796.
11.- Rees DD, Palmer RM, and Moncada S. Role of endothelium-derived nitric oxide in the regulation of blood pressure. Proc.Natl.Acad. Sci.USA. 1989; 86:3375-3378.
12.- Vázquez C B, Escalante B. Renal vascular interaction of angiotensin II and prostaglandins in renovascular hypertension. Journal of Cardiovasc. Pharmacol. 1999; 34 No. 1: 21-27.
13.- Ribeiro MO, Antunes G, Zatz R. Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. Hypertension. 1996; 20: 298-303
14.- Pollock DM, Polakowski J S, Divish B J, et al. Angiotensin blockade Reverses hypertension during long-term nitric oxide synthase inhibition. Hypertension. 1993;21: 660-666
15.- Fortepiani LA, E Rodrigo, Ortiz MC. Pressure natriuresis in nitric oxide-deficient hypertensive Rats: effect of antihypertensive treatments. J Am Soc Nephrol. 1999; 10: 21-27
16.- Cheng W, Oike M, Hirakawa M. Excess l-arginine restores endothelium-dependent relaxation impaired by monocrotaline pyrrole. Toxicology and Applied Pharmacology. 2005; 207:187-94.
17.- Singal A, Anjaneyulu M, Chopra K. Modulatory role of green tea extract on antinociceptive effect of morphine in diabetic mice. J Med Food. 2005; 8: 386-91.
18.- Baisch AL, Urban H, Ruiz AN. Endothelium-dependent vasorelaxing activity of aqueous extracts of lyophilized seeds of Casimiroa edulis (AECe) on rat mesenteric arterial bed. J Ethnopharmacol. 2004; 95:163-7.