

**ANTIHYPERTENSIVE EFFECT OF *Heimia salicifolia* (H.B.K.) ALKALOIDS.**

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**Summary**

In order to evaluate the effects produced by the alkaloids of leaves from *H. salicifolia* on the blood pressure of rats, four alkaloids from *H. salicifolia* were isolated by column and thin layer chromatography. The alkaloids' antihypertensive effects on angiotensin II-caused acute hypertension as well as their hypotensive effects on normotensive rats were studied. The alkaloid 3, the most abundant isolated alkaloid, turned out to be the most effective, while the other alkaloids showed a slight, non-significant vascular activity. Their mixture showed an additive effect, though slightly different from the alkaloid 3.

Key words *Heimia salicifolia*, quinolizidine alkaloids, arterial pressure, hypertension

*Heimia salicifolia* (HBK) Link, (Lithraceae) is a shrub which grows wild from Mexico to Argentina. In Mexico this plant is commonly known as "sinicuiche", and has been used in folk medicine of several countries for many medical purposes (1,2). The chemical analysis of *H. salicifolia* leaves showed the presence of quinolizidine alkaloids as: nesodine, lyfoline vertine and lythrine. Several investigations have proposed that these alkaloids possess cardiovascular pharmacological effects producing hypotension in dogs, cats and rats (3, 4). In our laboratory we have found that the aqueous extract from *H. salicifolia* leaves decreased the systolic blood pressure in normotensive rats, therefore, the present study was designed to investigate the hypotensive and antihypertensive activity of the isolated alkaloids from *H. salicifolia* in normotensive rats in the presence and absence of angiotensin II.

### Methods

Dried leaves of *H. salicifolia* were obtained from the Sonora Marketplace of Mexico City a marketplace specialized in selling medicinal plants. The identification was carried out by verified in the Herbarium of the Botanic Department of the Iztacala Faculty, National Autonomous University of Mexico (UNAM). The specimen deposited with the voucher number 41653, was authenticated by Edith Lopez Franco, Biologist in charge of the herbarium. The experiments were performed on male Wistar rats (300-350 g) housing them at 24 ±0.5°C with 12 h light/12 h dark photoperiod. They were fed laboratory diet and water *ad libitum*, Animals were randomly divided into 5-6 groups of six rats each. All procedures were conducted in accordance with Institutional ethical guidelines.

One kg of dried *H. salicifolia* leaves was washed, re-dried at 45 °C during 48 hours and ground in a container. Afterwards, 4 liters of petroleum ether (J.T. Baker) were added and the extract was obtained in 5 liters of methanol (J.T.Baker) during 24 hours. The detection of alkaloids was carried out by adding the Dragendorff reagent. Alkaloids were vacuum concentrated in a rotary evaporator (Buchi rotavapor model Mp60) until having a final volume of 50 ml, then the concentrate was dried at °C for 24 hours and added to 100 ml distilled water and acidified to pH 2.0 with 10% hydrochloric acid solution (Tec. Chem.), and filtered with aid Celite (Sigma Chem) and the precipitate washed with distilled water. The aqueous acidic filtrate was further defatted in a continuous extractor with 500 mL ethyl ether. The pH was adjusted to 9 with 28% ammonium hydroxide solution and the solution was extracted continuously with 200 mL chloroform. The chloroform extract was dried *in vacuo* at 40°C. The extract, dissolved in chloroform, was adsorbed on basic alumina (J.T.Baker) dried, and placed on the top of a column (2 X 70 cm) of basic alumina (J.T.Baker). Elution was with methanol-chloroform (1:1) and finally with methanol. The effluent collected in three fractions was examined for alkaloid composition by thin-layer chromatographic analysis. Each fraction was dried *in vacuo* and subsequently used for alkaloid isolation using column and thin layer chromatography. The solvent mixture used was chloroform-methanol (3:2) (5).

Rats were anesthetized with sodium pentobarbital (45 mg/Kg by i.p. injection), the femoral vein and carotid artery were isolated and cannulated for drug administration and blood pressure recording, respectively. The cannulae were filled with heparinized saline to prevent clotting. Blood pressure was recorded with a pressure transducer (Narco Byo-System mod. D1000B) connected to the arterial cannula. The signal from the transducer was electronically dampened and inscribed on a physiograph (mod. DPM-4B). Once the blood pressure was stabilized, dose-response curves to mixture alkaloids (1-5 mg/Kg) and each of the isolated alkaloids (1-2 mg/Kg) were carried out.

In other experiments acute hypertension was obtained by dose–response curve to angiotensin II (Sigma Chemical Co. (St. Louis, MO, USA) at a dose of 25 to 250 ng/kg, then, when the blood pressure returned to basal line, the mixture of alkaloids and /or the isolated alkaloids were administrated (2 mg/kg), and the dose-response curve to angiotensin II were carried out.

Statistical analysis: Data are expressed as the mean  $\pm$ ; the standard error of the mean. Groups were compared by the two-way ANOVA for unpaired samples, followed by the Tukey post hoc test. Statistical significance was set at  $p < 0.05$ .

### Results

The alkaloids isolated from one kilogram of *H. salicifolia* dried leaves averaged 0.310 g (0.031%). Four alkaloids were obtained when the extract was separated by thin layer chromatography and their Rf were considered. Table 1 shows the order of migration in silica gel.

Table 1 The Rf of *H. Salicifolia* isolated alkaloids by thin layer chromatography using the chloroform methanol (3:2) solvent mixture.

<b>Alkaloids</b>	<b>Rf</b>
<b>Alkaloid 1</b>	<b>0.92</b>
<b>Alkaloid 2</b>	<b>0.87</b>
<b>Alkaloid 3</b>	<b>0.55</b>
<b>Alkaloid 4</b>	<b>0.35</b>

The administration of the mixed *H. salicifolia* alkaloids to normotensive rats lead to a decrease in systolic blood pressure of  $20 \pm 7$  mmHg (Figure 1).

The increase in systolic blood pressure due to angiotensin II administration was dose-dependent, and reached its highest value at 100 ng/kg body weight of  $165 \pm 1.64$  mmHg which indicates an increase of  $37.7 \pm 1.64$  mmHg. This effect was partially blunted when rats were previously treated with 2 mg/kg of the *H. salicifolia* alkaloid mixture (Figure 2).

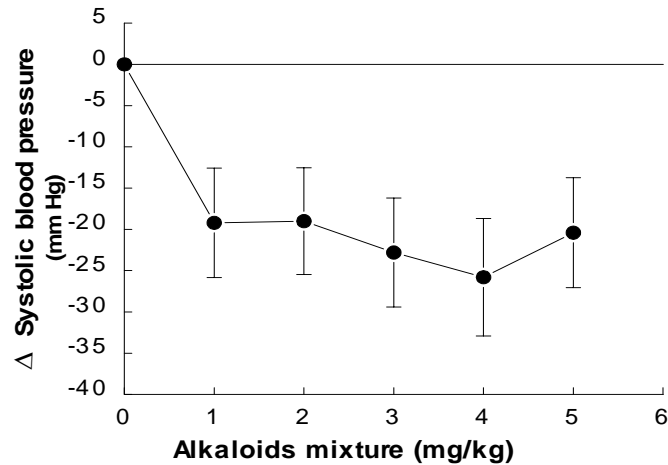


Figure 1. Effect of the alkaloid mixture on the systolic blood pressure of normotensive rats. A significant decrease of systolic blood pressure was observed after 1 mg/kg. Values are the mean  $\pm$  SEM (n = 5), \*p < 0.05 when compared vs the control group.

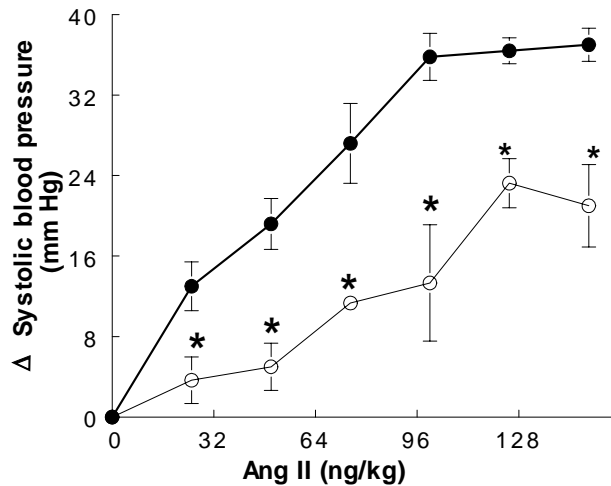


Figure 2. Effect of the alkaloid mixture on the dose-response curve of angiotensin II in rats. The alkaloid mixture (○; 2 mg/kg) blunted the increase in systolic blood pressure attributed to angiotensin II (●), this effect was observed at every point measured. n = 5. \*p  $\leq$  0.05

When the dose-response curve to each one of the isolated alkaloids was performed, a decrease of  $26.0 \pm 3$  mmHg and  $27 \pm 4$  mmHg in the systolic blood pressure was observed only with alkaloids 2 and 3 respectively (Figure 3). The other alkaloids did not show a significant decrease in systolic blood pressure (data not shown). On the other hand, the alkaloid 3 was more

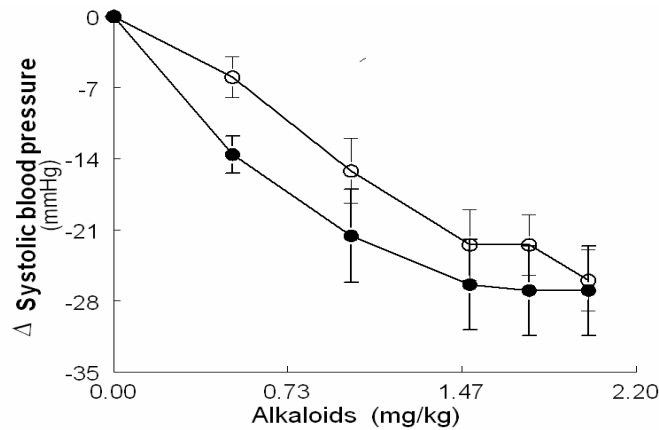


Figure 3. Effect of alkaloids 2 (○) and 3 (●) on the systolic blood pressure of normotensive rats. A more pronounced decrease relative to the control group was observed with the alkaloid 3 than with the alkaloid 2. Values are the mean  $\pm$  SEM (n = 5),  $p < 0.05$ .

Effective in decreasing the systolic blood pressure than alkaloid 2. In other experiments the effect of the isolated alkaloids on the dose-response curve to angiotensin II was studied and was observed that the alkaloid 3 blunted the maximum response to angiotensin II in 30% (Figure 4). The alkaloid 2 showed the same tendency than the alkaloid 3, but a milder effect (data not shown).

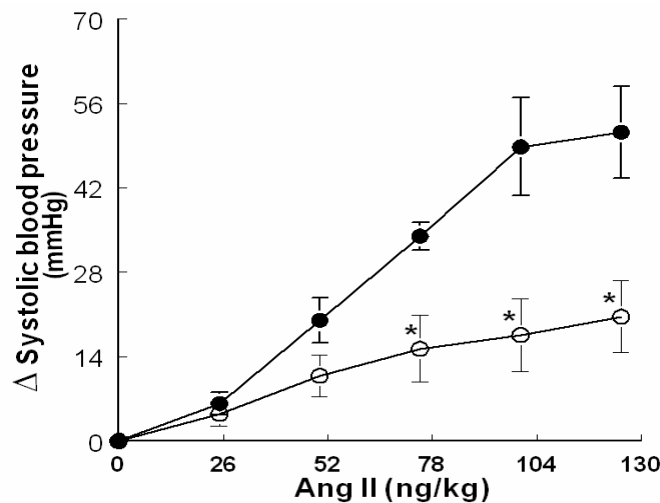


Figure 4. Effect of the alkaloid 3 on the dose-response curve of angiotensin II in rats. At a dose of 2 mg/kg, the alkaloid 3 (○) partially blunted the increase in blood pressure caused by angiotensin II (●). Values are the mean  $\pm$  SEM (n = 5), \* $p < 0.05$ .

### Discussion

This study shows that the alkaloid mixture isolated from *H. salicifolia* leaves decreased the systolic blood pressure of normotensive and hypertensive rats. The separation of the alkaloid mixture by thin layer chromatography produced four alkaloids. When these alkaloids were studied on normotensive rats, it was observed that only alkaloids 2 and 3 decreased the blood pressure, while the alkaloids 1 and 4 did not have significant effects. Our results show that the alkaloid 3 was the most abundant of the quinolizidine alkaloids present in *H. salicifolia* and that it has both hypotensive and antihypertensive effects in rats.

The renin-angiotensin system plays an important role in the physiological regulation of blood pressure (6). Angiotensin II causes peripheral vasoconstriction, aldosterone release, stimulation of sympathetic activity and cell growth by binding to its AT1 receptors (7). Hyperactivity of this system can lead to hypertension. Angiotensin II-caused acute hypertension is an often used experimental model in research. It has been previously shown that *H. salicifolia* alkaloids bind to nicotinic and muscarinic acetylcholine receptor (8), which suggests that their hypotensive and antihypertensive effects could be brought about through the interaction with endothelial muscarinic receptors, causing relaxation of vascular smooth muscle cells and hypotension by release of nitric oxide (9).

Our results show that the alkaloids isolated from *H. salicifolia* have both hypotensive and antihypertensive effects on anesthetized rats and that the alkaloid 3 is the most effective, blunting blood pressure increase after Angiotensin II administration, maybe through nitric oxide release from endothelial cells. These findings could offer new avenues to clinical application and to the development of new antihypertensive drugs.

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