

## Comparison of the Hypotensive Effect induced by the Ethanol Extract of *Albizia inopinata* G. P. Lewis in Normotensive and Chronically L-NAME Hypertensive Rats

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**SUMMARY.** The aim of this study was to characterize the hypotensive response induced by the aqueous fraction of the ethanolic extract of the leaves (AFL) of *Albizia inopinata* in normotensive and chronically L-NAME-hypertensive rats. AFL induced dose-dependent decreases in mean blood pressure (MAP) in both cases, however, hypotension was more pronounced in hypertensive rats. In isolated rat aortic rings, increasing concentrations of AFL were able to antagonize phenylephrine and KCl-induced contractions, however, IC<sub>50</sub> values for normotensive rats were significantly higher than that obtained for hypertensive rats. Furthermore, AFL-induced response was clearly endothelium-dependent since after removal of aortic endothelial cells and NO-synthase blockade, the relaxant response was significantly attenuated. The present work demonstrates that in normotensive and L-NAME-hypertensive rats, AFL lowers MAP, probably due to a decrease in peripheral resistances. The more pronounced hypotensive effect observed in hypertensive rats, is probably due to a strong vasodilation induced by AFL in these rats.

**RESUMEN** "Comparación del efecto de la hipotensión inducida por el extracto del etanol de *Albizia inopinata* G.P. Lewis en ratas normotensas y crónicamente hipertensas por el L-NAME". El objetivo del presente estudio fue caracterizar la respuesta hipotensora provocada por la fracción acuosa del extracto etanólico de hojas (AFL) de *Albizia inopinata* en ratas normotensas y hipertensas por el L-NAME (N-nitro-L-arginina metiléster). En las ratas normotensas, AFL provocó una disminución dosis-dependiente de la presión arterial, acompañada de un aumento de la frecuencia cardíaca. Ya en el caso de las ratas hipertensas, AFL provocó una hipotensión más aguda, en tanto que las modificaciones de la frecuencia cardíaca no fueron significativas. En los anillos de la aorta, concentraciones crecientes de AFL antagonizaron las contracciones provocadas por fenilefrina y KCl; por otro lado, los valores de CI<sub>50</sub> para ratas normotensas fueron significativamente superiores a los obtenidos en ratas hipertensas. El efecto vasodilatador de AFL parece ser claramente dependiente de la liberación de NO por el endotelio vascular. En conclusión, el presente estudio demuestra que AFL disminuye la presión arterial en ratas normotensas e hipertensas, debido probablemente a una disminución de la resistencia periférica total. El efecto hipotensor mayor observado en ratas hipertensas es causado probablemente por una vasodilatación potencializada, provocada por AFL.

### INTRODUCTION

*Albizia inopinata* (Harms) G.P. Lewis (Leguminosae) is a plant popularly known as "bordão de velho" and "casqueiro". In Brazil, the species *Albizia inopinata* is distributed sparsely throughout the country, but is especially abundant in the northeast. It is well reported in the literature that *Albizia chinensis* and *Albizia gummifera* exert hypotensive effects in cats and

guinea-pigs <sup>1</sup>, *Albizia lebbek*, *Albizia lebbekoides* are active in dogs <sup>2,3</sup>, whereas *Albizia odoratissima* has a diuretic activity in cats <sup>4,5</sup>.

Previous studies realized in our laboratory, in conscious unrestrained normotensive rats, demonstrated that the aqueous fraction of the ethanolic extract of the leaves (AFL) of *Albizia inopinata* (Harms) G.P. Lewis produced significant and dose-dependent hypotension associat-

**KEY WORDS :** *Albizia inopinata*, Hypotension, Hypertensive rats, Vasodilation.

**PALABRAS CLAVE:** *Albizia inopinata*, Hipotensión, Ratas hipertensas, Vasodilatación.

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ed with increases in heart rate and cardiac output, and with a strong reduction in total peripheral resistances <sup>6</sup>. In isolated rat aortic rings, increasing concentrations of AFL were able to antagonize the effects of phenylephrine and potassium chloride induced contractions. In addition, the hypotensive and vasorelaxant responses to AFL were significantly attenuated after nitric oxide (NO) synthase blockade <sup>6</sup>.

The aim of this study was to compare the hypotensive and vasorelaxant responses induced by AFL in normotensive and chronically L-NAME hypertensive rats and to investigate the mechanisms involved in the effects induced by AFL of *Albizia inopinata* in normotensive and chronically L-NAME-hypertensive rats, emphasizing the role of endothelium-derived NO and calcium mobilization on the relaxant response induced by AFL in the rat aorta.

## MATERIAL AND METHODS

### Animals

Male Wistar rats (200-350g) were used for all experiments. The animals were housed under conditions of controlled temperature ( $21 \pm 1$  °C) and exposed to a 12 h, light-dark cycle and had free access to food and tap water. Two groups of animals were used: normotensive rats, receiving tap water (free access) and L-NAME hypertensive rats, that were obtained by giving 0.5 mg/ml of N-nitro-L-arginine methyl ester (L-NAME) in drinking water for 6 days. Rat weight and water intake were measured and drinking water was changed each two days.

### Preparation of the aqueous fraction of the ethanol extract of the leaves of *Albizia inopinata* (AFL)

Mature leaves of *Albizia Inopinata*, collected from the district of Santa Rita, Paraíba (voucher specimen code Agra & Góis 3599) were dried at 40 °C in an oven and pulverized. The powder was extracted with 70% ethanol in water at room temperature (25-30 °C) for 72 h. The resulting extract was dried at 60 °C using a rotavaporator and the yield was *ca.* 22%. When required, this extract was dissolved in water, filtered and known volumes were dried to determine the concentration, as only 70% of the crude ethanol extract was water soluble. This factor was used to calculate the final concentration of AFL.

### Drugs

The drugs used were: sodium pentobarbital

(Cristália); heparin sodium salt (Roche); L (-) phenylephrine hydrochloride, sodium nitroprusside, acetylcholine hydrochloride, N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) hydrochloride (all from Sigma). The stock solutions were dissolved on distilled water and kept at 0 °C. All the drugs were immediately dissolved in distilled water or saline before the experiments.

The composition (mM) of the physiological salt solutions were as follows: Krebs's Henseleit Solution: NaCl 118.0, KCl 4.7, NaHCO<sub>3</sub> 25.00, CaCl<sub>2</sub>·2H<sub>2</sub>O 2.5, glucose 11.1, KH<sub>2</sub>PO<sub>4</sub> 1.2, and MgSO<sub>4</sub>·7H<sub>2</sub>O 1.2. Potassium chloride (80 mM) Solution: NaCl 57.0; KCl 80.0; NaHCO<sub>3</sub> 23.1, KH<sub>2</sub>PO<sub>4</sub> 1.1, MgSO<sub>4</sub>·7H<sub>2</sub>O 5.7, CaCl<sub>2</sub>·2H<sub>2</sub>O 2.5, glucose 11.0

### Direct blood pressure measurements in conscious unrestrained rats

Intra-aortic blood pressure was recorded in conscious freely moving normotensive and hypertensive rats by using a technique described elsewhere <sup>8</sup>. 24 h before experiments, rats were anesthetized with sodium pentobarbital (45 mg/Kg, i.p.) and polyethylene catheters were inserted into the lower abdominal aorta, via the left femoral artery, using a polyethylene catheter which consisted of piece of heat-stretched PE-10 fused to a PE-50 extension. Stretching of the PE 10 catheter minimized the risk of ischemia in the catheterized leg, without altering the arterial pressure signal <sup>8</sup>. Arterial blood pressure was measured by connecting the arterial catheter to a pre-calibrated pressure transducer (BLPR, WPI, Sarasota, FL, USA) for blood pressure recordings. Transducer was coupled to an amplifier recorder (TMB-4, WPI, Sarasota, FL, USA) and to a personal computer (Pentium 166 Mhz) equipped with an analog-to-digital converter board (CIO-Ad-Jr-16 WPI, Sarasota, FL, USA) using a CVMS software (WPI Sarasota, FL, USA). Data were sampled at 500 Hz. Beat-to-beat time series were generated and processed off-line on a personal computer. For each cardiac cycle, the computer calculated mean arterial pressure, and pulse interval (referred to as heart rate). In addition, a venous catheter was inserted into the inferior vena cava via the left femoral vein for the administration of drugs. Thereafter, both catheters were filled with heparinized saline solution and led under the skin to exit at the back of the neck. On the day of experiments, after cardiovascular parameters had stabilized, sodium nitroprusside (10 µg/kg, i.v.) was injected to check the patency of the venous catheter.

**Studies using isolated rat aortic rings**

Rat thoracic aortic rings (2-4 mm) were obtained of rats free from connective tissue and fat and suspended by platinum hooks for isometric tension recordings in a Kreb's-Henseleit solution maintained at 37 °C and gassed with a mixture of 95% O<sub>2</sub> and 5% CO<sub>2</sub>. The rings were allowed to equilibrate for 1 hour under a resting tension of 1 g. Mechanical activity was then recorded isometrically by a force transducer (FORT-10, WPI, Sarasota, FL, USA) coupled to an amplifier (TBM-4, WPI, Sarasota, FL, USA). Meanwhile, the bathing solution medium was changed every 15 min to prevent the accumulation of metabolites <sup>9</sup>.

**Statistics**

Except when otherwise specified, values are expressed as mean ± s.e.mean. Statistical analysis were performed by means of paired and unpaired Student's t-tests. Linear regressions were done by the least square method.

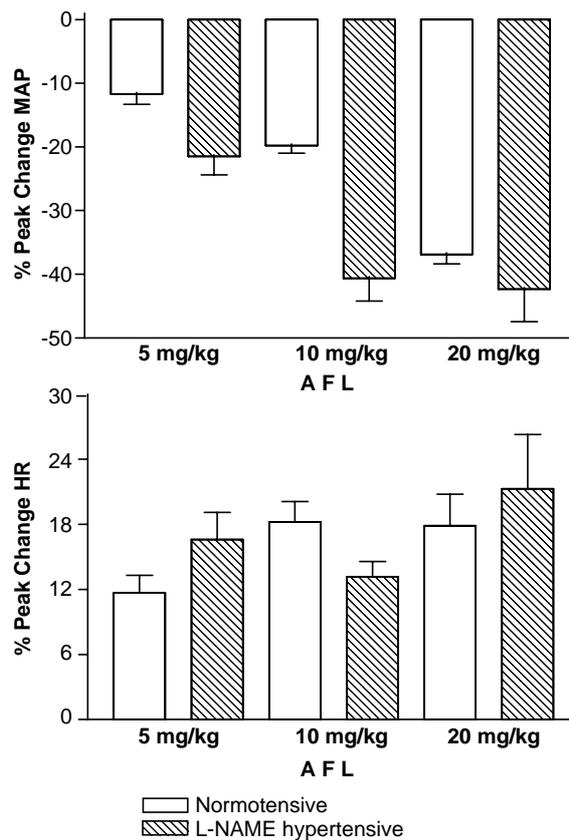
**RESULTS**

**Effect of AFL on mean blood pressure (MAP) and heart rate (HR) in conscious normotensive and chronically L-NAME hypertensive rats**

In conscious rats, baseline values of MAP and heart rate for normotensive rats (116±6 mmHg, 365±9 bpm, respectively) were significantly (p < 0.05) different from that for chronically L-NAME hypertensive rats (159±3 mmHg, 421±10 bpm, respectively). In normotensive rats, AFL (5, 10 and 20 mg/Kg, i.v., randomly) induced dose-dependent decreases in MAP (12±2, 20±1 and 37±1%) that were associated with increases in HR (13±3, 18±2, 18±3%). In L-NAME hypertensive rats, increasing doses of AFL induced a more significant decrease in MAP (21±3, 41±4, and 42±5%), nevertheless, changes in HR were not significantly modified (17±2, 13±1, and 21±5%) when compared to normotensive rats (Fig. 1). Hypotensive responses induced in both normotensive and hypertensive animals were rapid in onset and short-lasting (5 min).

**Effect of AFL on aortic isolated rings of normotensive and L-NAME hypertensive rats**

AFL (10, 20, 40, and 80 µg/ml), was able to antagonize phenylephrine (PHE, 1 µM) induced contractions in the presence of intact endothelium and in endothelium denuded aortic rings,

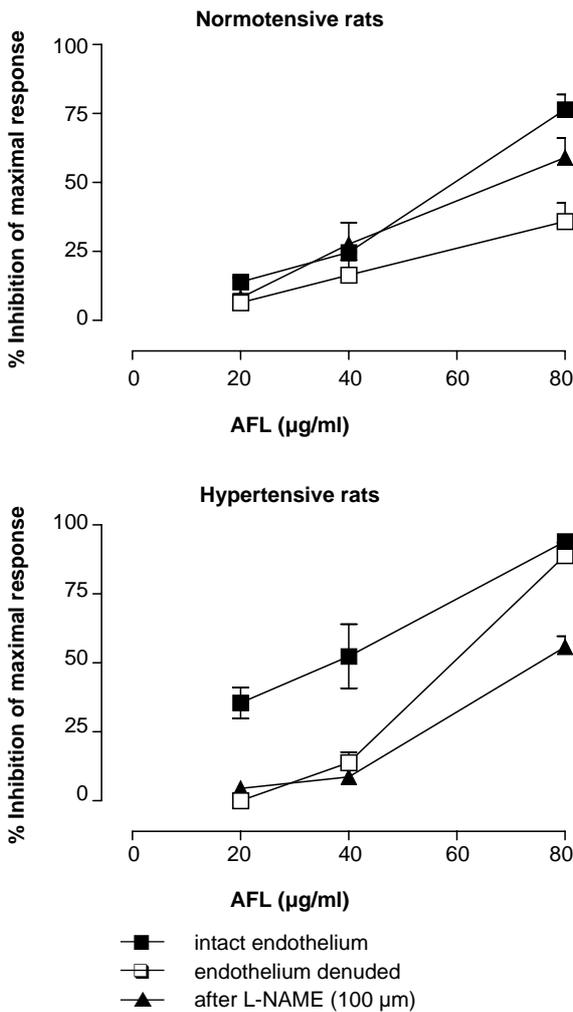


**Figure 1.** Bar graphs show peak changes in mean arterial pressure (MAP, %) and heart rate (HR, %), induced by the acute administration of increasing doses (i.v.) of AFL in conscious normotensive (n = 9) and L-NAME hypertensive rats (n = 13). Results are means ± s.e.m.

however, IC<sub>50</sub> values for normotensive rats were significantly (p<0,05) higher than that obtained with L-NAME hypertensive rats (Figure 2). The maximal relaxing response was obtained with AFL 160 µg/ml, which was not significantly different from that induced by AFL 80 µg/ml (results not shown).

Experiments also demonstrated that the response induced by AFL was clearly endothelium-dependent, since, after removal of aortic endothelial cells and NO-synthase blockade L-NAME (100 M), the relaxant response was significantly altered (Fig. 2).

Furthermore, in normotensive and hypertensive aortic rings, AFL (10, 20, 40, and 80 µg/ml) antagonized in a significant (p<0.05) and concentration-dependent manner (IC<sub>50</sub>= 54±6 and 26±1 µg/ml, respectively) KCl (80 mM)-induced contractions. However, IC<sub>50</sub> values for normotensive rats were significantly (p<0,05) higher than that obtained with L-NAME hypertensive rats (Fig. 3).

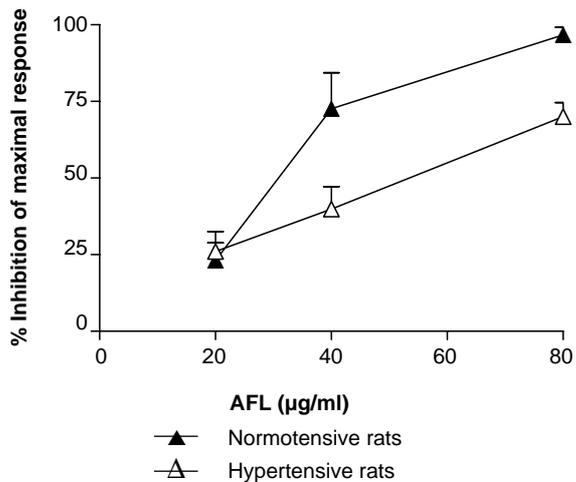


**Figure 2.** Line plot graph showing the vasorelaxant effect of increasing concentrations of AFL (20, 40 and 80 µg.ml<sup>-1</sup>) on isolated rat aortic rings intact, after endothelium removal and after acute NO-synthase inhibition with L-NAME (100 µM), pre-contracted with PHE (1 µM) in normotensive (n = 7) and L-NAME hypertensive rats (n = 7). Results are means ± s.e.m., \* p<0,05 vs intact endothelium.

## DISCUSSION

The major finding of this study is that in conscious normotensive and L-NAME hypertensive rats, the acute administration of AFL induces a short-lasting, dose-related hypotensive response, as a consequence of a decrease in total peripheral resistances.

We chose to measure hemodynamic parameters in conscious freely moving rats so as to minimize the influence of anesthesia and surgical stress<sup>10,11</sup>. On the other hand, we also used isolated aortic and atrial preparations, which are suitable to study specific effects, in the absence of neurohumoral influences.



**Figure 3.** Line plot graph showing the vasorelaxant effect of increasing concentrations of AFL (20, 40 and 80 µg.ml<sup>-1</sup>) on isolated rat aortic rings intact endothelium pre-contracted with KCl (80 mM) in normotensive (n = 7) and L-NAME hypertensive rats (n = 7). Results are means ± s.e.m., \* p<0,05 vs hypertensive.

We hypothesized a relaxant activity of AFL on vascular smooth muscle, which could be verified using isolated aortic rings. In these preparations, AFL antagonized in a concentration-dependent manner phenylephrine- and KCl-induced contractions. It is well established that NO is a major endothelium-derived relaxing factor, both *in vivo* and *in vitro*<sup>12</sup>, and that the release of NO from endothelial cells leads to relaxation of vascular smooth muscle cells and plays a critical role in the maintenance of vascular tone<sup>12,13</sup>. In order to determine whether part of the relaxant effect produced by AFL in isolated aortic rings could be due to NO release, we performed experiments in intact aortic preparations incubated with increasing concentrations of L-NAME, a competitive inhibitor of NO-synthase<sup>13</sup>. Under these conditions, in both normotensive and hypertensive rats, AFL-induced smooth muscle relaxation was significantly attenuated, but not completely abolished. Furthermore, in preparations in which the endothelium was mechanically removed, the AFL-induced vasorelaxant action was significantly attenuated. Interestingly, in both L-NAME-incubated or endothelium-denuded aortae, the inhibition of phenylephrine-induced contractions was of similar magnitude. These results indicate that relaxing factors released by the endothelium, mostly NO, may play an important role in the vasorelaxant response to AFL in aortic preparations from normotensive and hypertensive rats.

In intact aortic preparations from normotensive rats, we found that AFL was able to antagonize, in a concentration-dependent manner, KCl- and phenylephrine-induced contractions with a similar potency. Nevertheless, in aortic rings from L-NAME hypertensive rats, AFL was more potent to antagonize KCl-induced contractions. Furthermore,  $IC_{50}$  values obtained for normotensive rats were significantly ( $p < 0,05$ ) higher than that obtained with L-NAME hypertensive rats. It is well known that KCl induces smooth muscle contraction through activation of voltage-dependent calcium channels and subsequent release of calcium from the sarcoplasmic reticulum<sup>14</sup>, whereas phenylephrine-induced vasoconstriction is mediated by the stimulation of G-protein coupled to  $\alpha$ -adrenoceptors<sup>15</sup>. In both cases, the major resulting effect is an increase in the intracellular calcium concentration through calcium entry. We therefore suggest that, in normotensive rats, the residual vasorelaxant effect observed after endothelium removal may be due to a NO-independent mechanism, possibly linked to a calcium mobilization antagonism. They further suggest that in aortic rings from hypertensive rats, the main mechanism involved in the relaxant activity is a calcium-channel (probably L type) blockade activity.

Finally, in conscious rats, AFL-induced hypotension was accompanied by tachycardia. Pilot experiments have shown that the hypotensive response to a pure vasodilator agent, sodium nitroprusside, in a dose (8  $\mu$ g/kg) which

produces a fall in MAP similar to that induced by 5 mg/kg of AFL, is followed by an intense reflex tachycardia ( $+130 \pm 10$  bpm,  $n = 6$ ). Comparing these data to those obtained with AFL, it is possible to suggest that even at relatively low doses, the extract has an additional negative chronotropic effect, which tends to oppose the hypotension-induced reflex tachycardia<sup>6</sup>.

In conclusion, the present study, using a combined *in vivo* and *in vitro* approach, demonstrates that the aqueous fraction of the ethanolic extract of the leaves of *Albizia inopinata* (Harms) G.P. Lewis lowers arterial pressure in rats through a decrease in peripheral vascular resistance. The more pronounced hypotensive effect in hypertensive rats is probably due to a strong vasodilation induced by AFL in these rats, as a consequence of a calcium-channel blocking activity. Furthermore, *in vitro* studies suggest that AFL-induced vasodilation in normotensive as well as in chronically L-NAME hypertensive rats is, at least in part, secondary to the release of endothelium-derived NO. However, additional experiments are necessary to clearly elucidate the mechanisms involved in AFL-induced hypotension in both, normotensive and chronically L-NAME hypertensive rats.

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