VASORELAXATION BY THE AQUEOUS EXTRACT OF *JULIANA ADSTRINGENS* ON RAT AORTIC RINGS

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Summary

In this paper, we study the effects of *Juliana adstringens* (JA) aqueous extract on the vasomotor reactivity, of rat aortic rings (with or without a functional endothelium). Juliana adstringens is a plant native to central and southern Mexico, where it grows in the low deciduous tropical forest, its name in Nahuatl is cuachalalatl. Isolated aortic thoracic rings from male rat, with and without endothelium, exposed to JA increased the resting tension in a dose dependent response. A reproducible cumulative concentration-response curve to norepinephrine (NE) ($3.1x\ 10^{-8}$ to $3.1x\ 10^{-5}$ M.) was shifted to right in presence of JA extract (1ml, 20 % w/v). In presence of nitric oxide synthase inhibitor N ώ-nitro-l-arginine methyl ester (L-NAME 30 μM) 30 minutes before the NE dose-response curve, the effect of JA was inhibited. The addition of JA, induced relaxation on NE precontracted aortic rings with endothelium. In presence of L-NAME (30 μM) was completely inhibited the relaxant effect induced by the JA extract. JA inhibited the relaxation induced by acetylcholine (ACh) on NE precontracted aortic rings with endothelium; atropine added before NE contraction, did not block the relaxant effect of JA extract. Our results suggest that nitric oxide mediated the relaxation induced by JA on aortic rings.

Key Words: Vasodilatation, nitric oxide (NO), N ώ-nitro-l-arginine methyl ester (L-NAME), Juliana adstringens extract, natural products.

Juliana adstringens Schltdl. (JA) is a native plant that grows in the low deciduous tropical forest of central and southern México; its name in Nahuatl is cuachalalatl. The cortex and roots of the plant, had been used in traditional medicine as antiseptic in skin damage, to harder the gum of the mouth, and for gastric ulcer. A methanolic extract of the stem bark of JA shows an inhibitory effect of gastric ulcers in rat [1]. The purpose of this work was to study the action and mechanism of aqueous extract of JA on aortic rings from male rat.

Methods

All experiments were done on the thoracic aorta [2] from male Wistar rat (250 – 300 g) from the animal facilities of the School of Medicine. They were maintained in a 12/12 hours dark-light period, with free access to rat pellet and water. All procedures were conducted according to the National Institutes of Health Guide for the Care of Laboratory Animals. The thoracic aorta was cleaned of fat and connective tissue, under microscopic guidance, and cut into segments ca. 5 mm wide. In some rings, the endothelium was removed by gently rubbing the inner surface with a glass rod. The aortic rings were placed in tissue chambers containing 10 ml of Krebs-bicarbonate solution, of the following composition (mM): Na^+ (142); K^+ (5.88); Ca^{2+} (2.5); Mg^{2+} (1.8); Cl^- (125.22); HCO_3^- (24.9); SO_4^{--} (1.18); $H_2PO_4^-$ (1.18); and dextrose (5.5), at pH 7.4 and 37 ^{o} C, bubbled with 95 % O_2 + 5 % CO_2. Isometric tension was recorded by suspending the preparation between two stainless steel hooks one of which was attached to the bottom of the chamber and the other to an isometric myograph transducer. An initial tension of 1 g was applied to the preparation and the vessel allowed to equilibrate for 1 hr before the experiment. After equilibration period, the contractile response induced by norepinephrine (10^{-6} M) was measured in all the aortic rings. Acetylcholine (10^{-5} M) was added to assess the integrity of the endothelium [3]. The values were expressed as the percentages of the maximum contractile responses to norepinephrine (10^{-6} M). Chemicals were of laboratory grade from various commercial sources.
Juliana adstringens aqueous extract was obtained adding 20 g of chopped cortex of the plant stem to 100 ml of boiling bidistilled water. All doses of JA are expressed in terms of volume extract.

*Statistical analysis***.** Data from JA and control assays were expressed as the mean ± standard deviation (SD). Significant differences among control and experimental groups were evaluated using Student's t-test. Differences were considered statistically significant when the p value was less than 0.05.

Results

Effects of the *Juliana adstringens* extract on the resting tension.

The first series of experiments evaluated the effect of the *Juliana adstringens* extract on the resting tension of the aortic rings (endothelium intact or denuded aorta). The rings were exposed to an increasing concentration of the *Juliana adstringens* extract (0.1 to 1.6 ml), JA increased the resting tension of aortic rings in a dose-response relationship. Compared with endothelium intact rings, denuded rings developed smaller tension in response to JA extract ($p < 0.05$) (Fig. 1).

Effects of *Juliana adstringens* extract on norepinephrine cumulative concentration-response curve.

In endothelium-intact rings, a cumulative concentration-response curve to norepinephrine (10^{-8} to 10^{-5} M.) was constructed as a control response. In presence of *Juliana adstringens* extract (1 ml) added to the tissue bath 15 min before, the NE dose-response curve was shifted to the right, developing less tension in response to each of the NE concentrations compared with the NE control curve ($p< 0.001$) (Fig. 2). The inhibitory effect of Juliana adstringens extract was reverted in presence of nitric oxide synthase inhibitor N ώ-nitro-l-arginine methyl ester (L-NAME 30 μM) [4] added to the tissue bath 30 minutes before the NE dose-response curve. The norepinephrine responses returned to the control values, except with the highest dose (NE 10^{-5} M). The inhibitory effect of JA extract on dose-response curve to NE was reverted with L-NAME (Fig. 2).

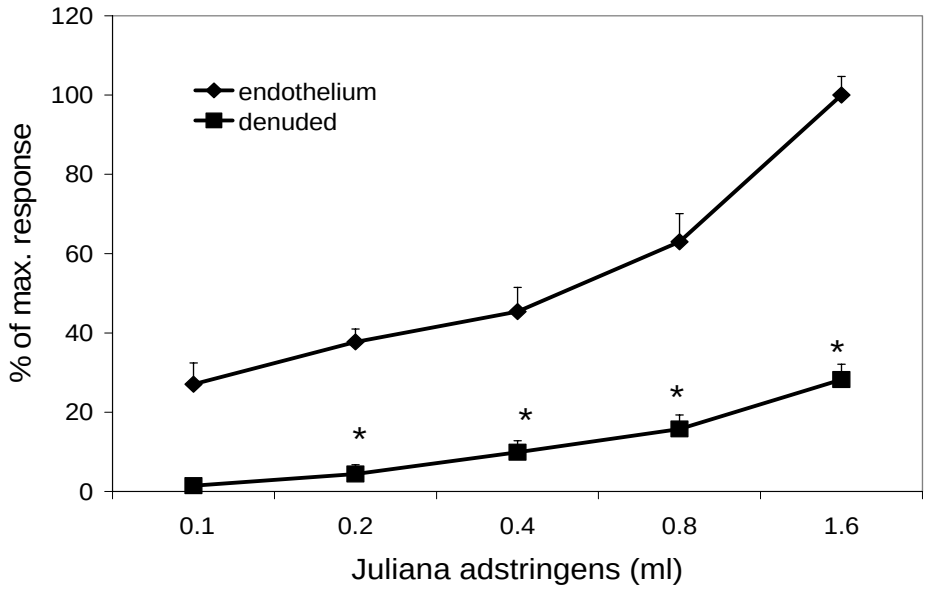

Figure 1. The figure shows cumulative concentration–response curves to the *Juliana adstringens* aqueous extract (20 %) on rat aortic rings with or without endothelium. Data are expressed as percent of the maximal tension induced by JA and represent the mean ± S.D. n = 9 ($p<0.05$). These responses denoted significant differences between the aortic rings with and without endothelium.

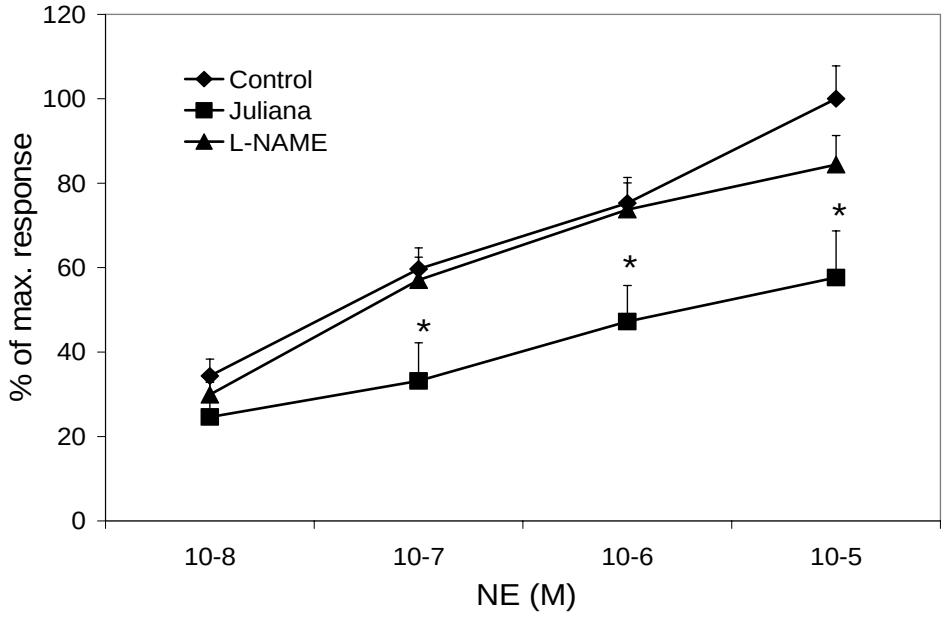

Figure 2. The graphic shows the inhibition induced by *Juliana adstringens* extract on cumulative concentration–response curves to NE in rat aortic rings, in absence or in presence of JA extract, and in presence of L-NAME (30μM). Data represent means ± S.D. n = 12 rings. Data are expressed as a percentage of the agonist maximum contraction obtained from control curve ($p<0.005$). The dose-response curve is shifted to the right and the maximum contraction is smaller in aortic rings with *Juliana adstringens*, this effect reverts with L-NAME.

Effect of *Juliana adstringens* extract on aortic rings precontracted with norepinephrine.

Cumulative concentration–response curve to *Juliana adstringens* extract (0.1, 0.2, 0.4, 0.8 and 1.6 ml) were performed on norepinephrine-precontracted aortic rings with endothelium. As shown in Fig. 3, concentrations of the JA extract induced relaxant responses, which were dependent of concentration (n = 9). After completing the concentration–response curve the rings were washed several times during 30 min with Krebs solution.
In order to investigate possible desensitization effect, a second cumulative concentration–response curve to *Juliana adstringens* was made on norepinephrine-precontracted aortic rings. Small doses of JA (0.1 ml) induced an additional tension. Whereas doses of JA above 0.4 ml, induced smaller relaxations compared with the first curve.

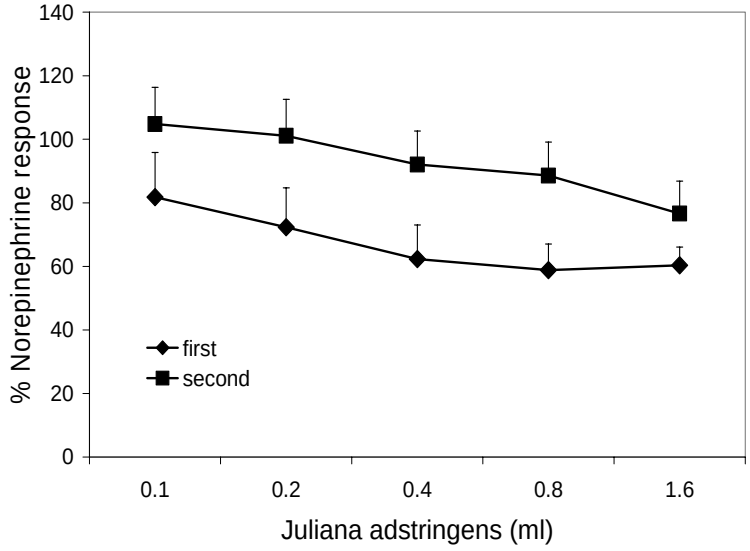

Figure 3. Effect of the Juliana adstringens aqueous extract on norepinephrine (NE)-precontracted aortic rings in two times. Relaxations are expressed as the percent of the maximum tension developed in response to NE (10^{-6} M) All data are expressed as means ± S.D. n = 9 (p<0.05).

The addition of L-NAME (30 μM) to the tissue bath solution 30 minutes before norepinephrine contraction reverted completely the relaxant effect induced by *Juliana adstringens* extract. Low doses of the extract developed an additional tension instead of the relaxant response (Fig. 4); these findings clearly indicate that nitric oxide (NO) is involved in the relaxing response of JA extract.

In another set of experiments
Aortic rings precontracted with KC l (77mM), exposed to JA extract (1 ml), no relaxing effect was observed. (Data is not shown). The relaxation induced by ACh (10^{-5} M) on aortic preparations precontracted with NE was inhibited by the addition of *Juliana adstringens* extract (1 ml) before NE contraction. In contrast, ACh (10^{-5} M) added to the bath, before JA extract (1ml), in preparations precontracted with NE, did not inhibit the JA relaxation. (Data is not shown).

In order to investigate if *Juliana adstringens* extract, acts on muscarinic receptors, we used two sets of rings. In the first set of aortic rings, we added atropine (20 μM) to the tissue bath 15 min before NE contraction. The addition of ACh (10^{-5} M) did not develop any relaxation. On the contrary in a second set of aortic rings, atropine added also before NE contraction, did not significantly inhibit the relaxant effect of JA extract (Fig. 5).

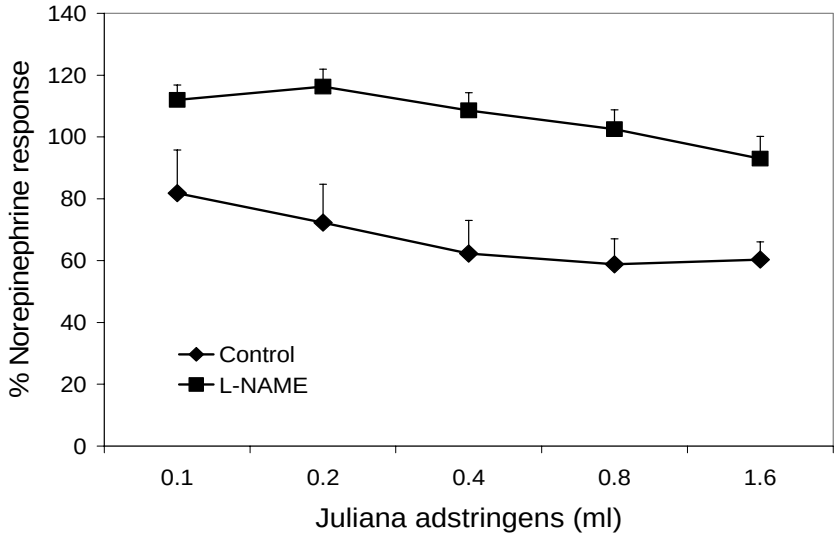

Figure 4. The graphic shows the effect of the Juliana adstringens aqueous extract on norepinephrine -precontracted aortic rings, control and in presence of L-NAME (30μM). All data are expressed as means ± S.D. n = 12 ($p<0.05$). The relaxation induced by JA, is reverted by L-NAME.

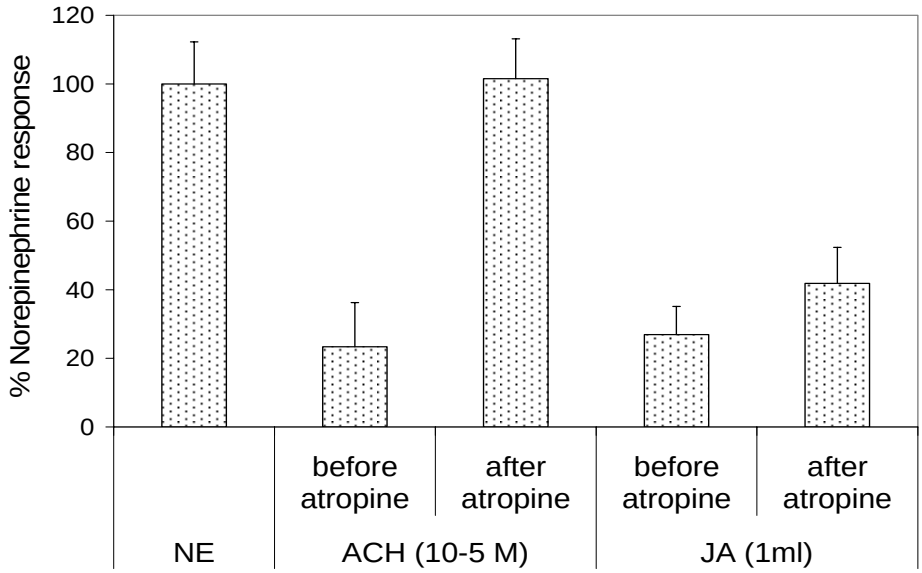

Figure 5. The graphic shows the effect of atropine (20 μM) before ACh (10^{-5} M) and JA (1ml) on NE precontracted aortic rings. Data are expressed as percent of NE response and represent the mean ± S.D. of n = 6. Atropine did not significantly inhibit the relaxation-induced by JA.

The addition of propranolol (20 μM) a beta-receptor inhibitor to the bath, before NE contraction had no significant effect on the relaxation induced by the JA extract.

Discussion

A useful approach to test the effects of plant-derived remedies on the cardiovascular system is to analyze, in vitro, the effects of a crude plant extract on the vasomotor reactivity of isolated vascular segments. In the present study is analyzed the effects of the aqueous extract of *Juliana adstringens* on the reactivity of rat aortic rings, with or without endothelium.
The aqueous extract of JA, increase the basal tension in endothelium or denuded aortic rings from male rat, in a dose-response relation. The basal tension significantly increased in rings with endothelium. This result suggests an influence of the endothelium on the release of some vasoactive compound and or that the JA extract decrease the basal liberation of nitric oxide by the endothelium. The finding that the treatment of vascular rings with inhibitor of NO synthase causes an increase in the potency of NE contraction supports this idea. [5] [6]

In presence of JA extract, the NE cumulative dose-response curve is shifted to the right and the amplitude of the tension developed by the aortic rings decrease significantly. The addition of nitric oxide synthase inhibitor (L-NAME) [4] before JA extract reverted completely the inhibitory effect of JA on the NE concentration-response curve.

The endothelium-dependent relaxation induced by the JA extract on rings precontracted with NE, also was completely reverted by L-NAME [4] and low doses of JA developed an additional tension over NE contraction instead to develop a relaxation. These findings clearly indicate that endothelium NO synthesis/release are involved in the vasodilatation induced by *Juliana adstringens* extract. We have proposed that the relaxation induced by JA extract is the result of an activating production and release of NO so full, that any additional activation is precluded, by the contrary a second application of JA probably developed a super sensitivity to the vasodilators, which resemble a condition of maximal NO inhibition [5]

The increase in tension, induced by low concentrations of the extract in a second application, suggests that it contains some vasoconstrictor compound.

The results showed that JA extract acts through nitric-oxide pathway in the vascular smooth muscle probably activating a receptor-operating channel [7, 8] since the JA extract added on KCl precontracted aortic rings did not develop any relaxation. Moreover, that JA extract, acts via other receptors different from muscarinic receptors since atropine did not block the relaxation induced by JA [9].

The interaction between JA and ACh relaxant effect showed that, ACh fail to produce any relaxation in presence of JA. In addition, ACh did not inhibit the relaxant effect of JA. It means that both substances did not act by competence and that the stimulation on the liberation of NO took place probably on the intracellular pathway.

Addition of a beta-receptor inhibitor (propranolol) to the bath before NE contraction, had no effect on the relaxation induced by the JA extract, it means that β adrenergic receptor do not interfere with the JA relaxation.

Acknowledgments

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