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# Vasorelaxant Effect of a *Baccharis trimera* Infusion on Precontracted Rat Aortic Rings

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*Baccharis trimera* (Less.) DC is a South American plant that in folk medicine is considered to produce reduction in blood pressure. One aspect of this putative effect is the vasorelaxation. The aim of this work was to evaluate the ability of a *B. trimera* extract to relax rat aortic rings precontracted with noradrenaline. As the infusion is the usual way of intake of this plant, an infusion of *B. trimera* was prepared using 100g of the plant (leaves) boiled in water, frozen and lyophilized. Working solutions were prepared using different concentrations of the dried extract diluted in Krebs Henseleit solution. It was proved that the infusion relaxed the aortic rings in a dose dependent manner 100 minutes after adding the extract to the bath. Considering as 100% the maximum contraction achieved with noradrenaline, a relaxation of 101.1±2.3% was observed with the highest dose of the infusion used in these experiments (0.32mg/mL). While in control rings relaxation was 12.9±2.4%. In aortic rings denuded from endothelium the percentage of vasoralaxation did not show statistically significant differences when compared to intact rings. These data support the hypothesis of a vasorelaxant effect of this plant and constitutes the first approach to the scientific basis of a potential antihypertensive effect.

**Keywords:** Hypertension, Cardiovascular, Traditional medicine, Meso-and Southtern American, *Baccharis trimera*, Vasorelaxation, Aortic rings.

*Baccharis trimera* (Less.) DC., popularly known as "Carqueja" is a widely distributed South American plant [1a,b, 2], commonly consumed as an infusion. From the ethnomedicinal point of view, it is believed to have many beneficial properties [1a, 2, 3], although those properties are weakly sustained on scientific basis. Two of their alleged effects are related to vascular issues i.e. the reduction of blood pressure and the benefic effect on erectile dysfunction. Chloroform extracts from the plant have shown to induce vascular smooth muscle relaxation in strips of rat portal vein [3]. Aqueous extracts have shown to relax the guinea pig corpus cavernosum [4]. Nevertheless, there are no scientific communications supporting its effect in arterial smooth muscle or related tissues to the reduction of blood pressure. Phytochemical analyses have revealed the presence of different constituents as terpenes (mono, di & tri) [3], flavonoids and saponins [5]. Flavonoids have been extensively reported as vasorelaxant and different mechanisms of action have been proposed [6-13]. The aim of this work was to evaluate the arterial vasorelaxant effect of an infusion of *B. trimera* in aortic rings precontracted with noradrenaline and its dependence with the presence of endothelium. This study constitutes a first approach to the scientific basis of a potential antihypertensive effect of *B. trimera*.

The infusion of *B. trimera* induced vasorelaxation of NA precontracted aortic rings in a dose-dependent manner (Figure 1). The calculated vasorelaxation (measured 100 min after the addition of the infusion) was in percentage 17±3.7% for the dose of $3.2x10^{-3}$mg/mL (n=5), 73.8±24.2% for the dose of $3.2x10^{-2}$mg/mL (n=7) and 101.1±2.3% for the dose of 0.32 mg/mL (n=6). The main components identified in the "Carqueja" infusion studied were polyphenols belonging to the chlorogenic acid family as well as quercetin glycosides, free quercetin and methyl quercetin

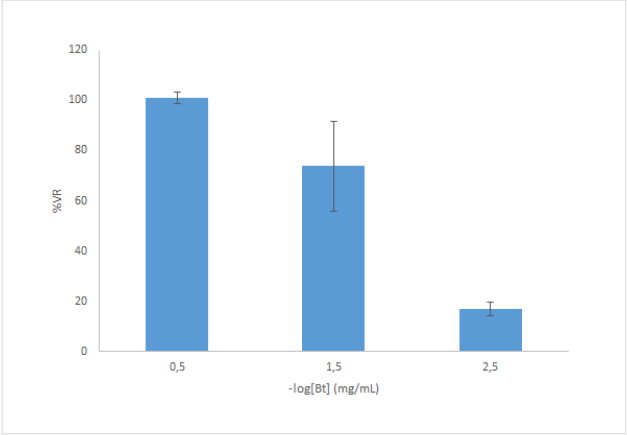


**Figure 1:** Vasodilatory profile of a *B. trimera* infusion on endothelium intact aortic rings precontracted with NA. Figure shows three different concentrations (in negative logarithmic values). VR= vasorelaxation.

flavonoids, like 3-O-Methyl quercetin and isorhamnetin. The identification was performed based on the retention times of pure standards and the matching of their UV spectra with the eluting compounds. A typical HPLC profile is shown in Figure 2.

The temporary course of tension of an intact aortic ring treated with the highest dose of the infusion and a control aortic ring are shown in Figure 3A and 3B, respectively. In control rings the percentage of vasorelaxation (100 min after the bolus of KH) was 12.9±2.4%. Statistical analysis showed very significant differences in relaxation between treated rings respect to control when challenged with 0.32 and $3.2x10^{-2}$mg/mL of the infusion (Figure 4).

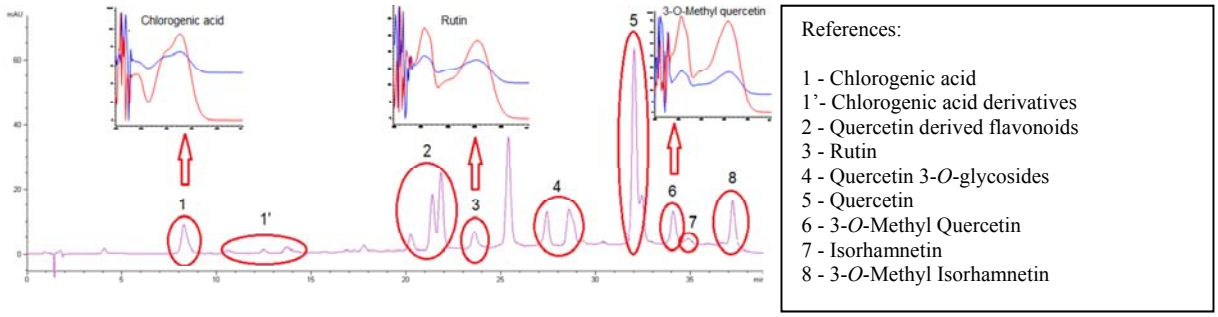


**Figure 2:** HPLC-DAD Chromatogram of the *B. trimera* infusion recorded at 358nm and peak assignments of the run.

In order to test the vasorelaxant response, nine different doses of ACh (from $1x10^{-5}$ to $1x10^{-9}$ M) were used. For these concentrations the percentage of vasorelaxation ranged from 0 to 80% (Figure 5).

Endothelium dependence of relaxant responses was tested in aortic rings whose endothelial layer was removed. For this purpose, the dose of the infusion that produced the maximal vasorelaxation in the dose-response curve (0.32mg/mL) was used. When vasorelaxation data from intact and denuded aortic rings were compared, no significant changes were observed (data not shown).

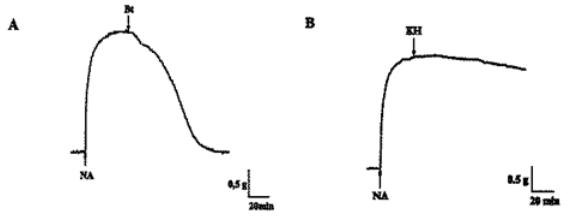


**Figure 3:** Representative recordings of vasorelaxation in aortic rings treated with an infusion of *B. trimera*. (A) and control rings (B).
NA: noradrenaline. Bt: Infusion. KH: Krebs Henseleit solution

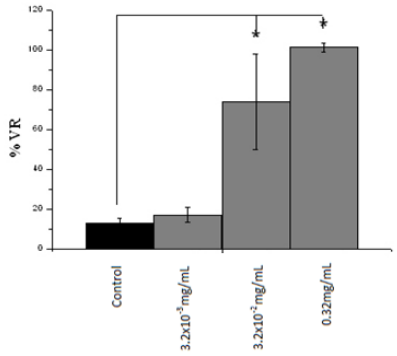


**Figure 4:** Vasorelaxation responses to different concentrations of an infusion of *B. trimera* on precontracted aortic rings. Figure shows the percentage of vasorelaxation with different dilutions; $3.2x10^{-3}$mg/mL, 3.2 $x10^{-2}$mg/mL, 0.32mg/mL. Control rings were precontracted and treated with KH. Statistical analysis reveals significant differences between the concentrated solutions respect to control (*).

The vasorelaxation observed in this work was dose-dependent. When rings were challenged using a concentration of $3.2x10^{-2}$mg/mL of the infusion the SD of the mean vasorelaxation value was higher than with the other concentrations (Figure 1). This finding let us think that this dose constitutes a point of inflection. Intermediate doses should be assayed in order to clarify the vasodilator profile.

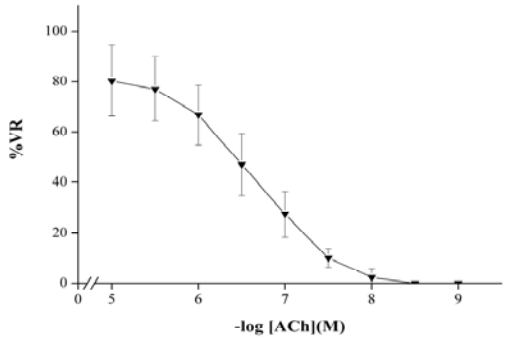


**Figure 5:** Dose-response curve of ACh in intact precontracted aortic rings (n=9). Vasorelaxation is proportional to the concentration of ACh expressed in negative logarithmic values.

Different types of compounds have been reported in *B. trimera* i.e. diterpens [2], saponins, chlorogenic acid and flavonoids [4]. Diterpens were identified as the active vasorelaxant constituents of *B. trimera*, blocking the smooth muscle contractions induced by extracellular calcium, in KCl depolarized strips of rat portal vein [2]. In the extracts studied, no diterpenes were detected but the presence of polyphenols from the chlorogenic acids family and quercetin flavonoids either glycosidated or not, was confirmed (Figure 2).

Both groups of polyphenols have been widely reported as protective compounds for the vascular system and there is abundant literature about their vasorelaxant effect in rat aortic vascular smooth muscle [5, 6, 8, 9,10, 12]. Different mechanisms of action have been proposed for the vasorelaxant effect of polyphenols and flavonoids. Some authors described an endothelial dependent mechanism trough the NO-GMPc pathway [5, 8]. Other authors found endothelium independent mechanisms which involve the opening of $Ca^{+}$ activated potassium channels and ATP sensitive potassium channels [5, 10], blockade of $Ca^{+}$ channels, reduction of sarcoplasmic reticulum $Ca^{+}$ release [5] and inhibition of protein kinase C [6]. Zhu *et al*. described both, endothelium dependent and independent mechanism of action with different doses [5].

When the endothelial layer was removed, no changes in vasorelaxation were observed for the highest dose of the infusion of *B. trimera* tested in this work. It seems that the mechanism of action did not depend only on the presence of an intact endothelium. As the vasorelaxant properties were observed also in the absence of endothelium but with a slow kinetics, an independent mechanism from endogenous NO and other endothelial pathways is also occurring.

These results demonstrate the arterial smooth muscle vasorelaxant properties of the infusion of *Baccharis trimera* (Less.) DC. towards rat aortic rings, which are likely to be due to endothelium dependent and independent mechanisms. This effect might correlate with the fall in blood pressure referred in folk medicine. However, further studies are needed to determine the mechanism underlying in the vasorelaxant response within the experimental conditions described here, and the individual influence of the identified polyphenols in the observed bioactivity.

## Experimental

***General:*** This investigation was performed in accordance to the Guide for the Care and Use of Laboratory Animals (NRC/USA/1996) and Comisión Honoraria de Experimentación Animal (CHEA), UdelaR Uruguay.

***Chemicals:*** Noradrenaline bitartrate (NA) was purchased from Hospira Laboratories (Levofed ®), acetilcholine (ACh) was purchased from Sigma (St. Louis, MO, USA).

***Preparation of the extract:*** Plant material was purchased at an herbal store ("Botica del Señor", Montevideo, Uruguay) and its authenticity was checked by Professor Eduardo Alonso Paz from the Herbarium "Arechavaleta" of Facultad de Química, UdelaR, Montevideo, Uruguay, where a voucher specimen was stored with the number MVFQ4359.

Aqueous extracts were obtained by adding 1000mL of boiling water to 100g of the plant material. The suspension was stirred for 30 min, then filtered and centrifuged (3000rpm, 5 minutes). The solution was frozen and lyophilized obtaining a yield of 8.4%.

***HPLC Analysis:*** A Hewlett Packard 1050 HPLC/DAD equipped with a METAPHOR®, ODS-3 (150 x 4.6) mm 5µm particle size. Gradient elution was performed and the elution profile was recorded at λ=280, 325, 354, 370 nm. The solvents employed were: solvent A: Water/Acetic acid (97.5:2.5 v/v), and solvent B: methanol/Acetic acid (97.5:2.5 v/v), at a 1.2mL/min flux. A stepwise gradient was employed for the infusion analysis. The first 20 min the solvent mixture was: solvent A/B in an 80:20 ratio; then 20-35 min A/B 50:50, followed by 35-39min A/B 40:60; and finally 39-45 min A/B 80:20. The eluting compounds were identified through their retention times and the fitting of the different UV spectra to those obtained in a house built polyphenol library using purchased compounds from Fluka®, Aldrich®, Chiron® and Extra Synthese®.

***Aortic ring preparation:*** Male Wistar rats (average weight 250 g) where heparinized and anaesthetized with sodium pentobarbital i.p. and then exanguinated. The descending thoracic aorta was dissected and placed in Krebs – Henseleit solution (KH) with the following composition: 20 mM $NaHCO_3$, 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM $NaH_2PO_4$, 1.2 mM $CaCl_2$, 5.6 mM glucose. The solution was gassed with a mixture of 95% $O_2$ and 5% $CO_2$ (carbogen). After the isolation of the aortic segment, the adventitia was carefully removed and the segment was cut into rings of 3-4mm length.

***Measurement of isometric vascular tension:*** Rings were mounted in tissue-bath chambers (Radnoti®) containing 30mL of KH maintained at 37ºC and gassed continuously with carbogen (pH= 7.4), by means of two parallel L -shaped stainless steel holders inserted into the lumen. One holder fixed the ring to the bottom of the chamber and the other was connected to a force displacement transducer to measure the isometric tension. Contractile responses were recorded using the computer data acquisition software DASY Lab®. After a resting tension of 2*g*, rings were allowed to equilibrate for 60 minutes.

***Experimental protocol:*** Aortic rings were precontracted with a bolus of 10µL of NA that yields a final concentration of 2µM in the bath. Once the contraction reached a plateau, the infusion of *B. trimera* was added in a bolus of 1000 µL. Three different doses were tested, $3.2x10^{-3}$mg/mL (n=5), $3.2 \times 10^{-2}$mg/mL (n=7) and 0.32 mg/mL (n=6). All the samples tested were dissolved immediately before their addition to the bath. In control rings a bolus of K-H solution with the same volume was added instead of the extract. As a positive control in precontracted rings, ACh was used in cumulative doses (from $1x10^{-9}$ to $1x10^{-5}$M).

Endothelium was mechanically removed by gentle rubbing the arterial lumen with a rough glass capillary tube, to investigate its involvement on the effects of *B. trimera* [13]. The effectiveness of this procedure was confirmed by the maintenance of the NA response (indemnity of smooth muscle), the absence of significant vasodilatation in response to ACh $1x10^{-9}$ to $1 \times 10^{-5}$ M) [14] and by the absence of the endothelial layer in histological preparations (data not shown). In such denuded aortic rings, a single dose of 0.32 mg/mL of *B. trimera* extract was tested.

***Data analysis:*** Responses (measured 100 min after adding the infusion to the bath) were evaluated as a percentage of the maximal contraction obtained with NA. Results are expressed as means ± SD. For comparison between groups a Mann-Whitney test was performed using Graph Pad in Stat version 3.01 for Windows (Graph Pad Software, San Diego - California). P values < 0.05 were considered significant.

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