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Vasodilatory properties of *Solanum crispum* Ruiz & Pav. a South American native plant

[Propiedad vasodilatadora de *Solanum crispum* Ruiz & Pav. una planta nativa de América de Sur]

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Abstract: *Solanum crispum* Ruiz & Pav. (*S. crispum*) is a southern South American native plant that is usually used in traditional medicine for the treatment of symptoms associated with both, acute and chronic ailments. Enema and infusion of leaves and stems are used to treat fever, headache, inflammation and hypertension. In this study, we aim to assess the vasoactive effect of hydroalcoholic extracts of *S. crispum* on isolated rat aorta rings. The hydroalcoholic extract of *S. crispum* induced a vasodilatory effect (42.6 ± 4.1%) in aortic rings precontracted with phenylephrine (0.1 µM). The aortic relaxation was largely endothelium-dependent and mediated by nitric oxide (NO). The endothelium- and NO-dependence was demonstrated by a drastic fall in the dilatation induced by the extract when the endothelium was removed (10.6 ± 2.3%) and when nitric oxide synthase (NOS) was inhibited (12.3 ± 2.5%) by nitro-L-arginine (L-NNA). This result supports the popular use of *S. crispum* in the treatment of hypertension that may be due, at least in part, to the vasodilator effect of one o more compounds present in their leaves and stems. Further studies should be performed to clarify this phenomenon.

Keywords: *Solanum crispum*, *Solanum berteroanum*, *Solanum ligustrinum*, *Solanum tomatillo*, *Witheringia berteroana*, rat aorta

Resumen: *Solanum crispum* Ruiz & Pav. (*S. crispum*) es una planta nativa de América del Sur meridional que se utiliza generalmente en medicina tradicional para el tratamiento de los síntomas asociados con dolencias agudas y crónicas. El enema y la infusión de las hojas y tallos se utilizan para tratar la fiebre, el dolor de cabeza, la inflamación y la hipertensión. El objetivo de este estudio fue evaluar el efecto vasoactivo de un extracto hidroalcohólico de *S. crispum* en anillos aislados de aorta de rata. El extracto hidroalcohólico de *S. crispum* indujo un efecto vasodilatador (42,6 ± 4,1%) en anillos aórticos precontraídos con fenilefrina (0,1 µM). La relajación de la aorta fue en gran parte dependiente del endotelio y mediada por el óxido nítrico (NO). La dependencia de endotelio y NO se demostró por una caída drástica de la dilatación inducida por el extracto cuando el endotelio fue removido (10,6 ± 2,3%) y cuando se inhibió la óxido nítrico sintasa (NOS) (12,3 ± 2,5%) mediante nitro-L-arginina (L-NNA). Este resultado apoya el uso popular de *S. crispum* en el tratamiento de la hipertensión que puede ser debido, al menos en parte, al efecto vasodilatador de uno o más compuestos presentes en sus hojas y tallos. Se deben realizar más estudios para aclarar este fenómeno.

Palabras clave: *Solanum crispum*, *Solanum berteroanum*, *Solanum ligustrinum*, *Solanum tomatillo*, *Witheringia berteroana*, aorta de rata

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INTRODUCTION

Solanum crispum Ruiz & Pav. (*S. crispum)* is a southern South American native plant belonging to the *Solanaceae* family. The most widely used synonyms of *S. crispum* include *Solanum berteroanum* (J.Rémy) Phil., *Solanum ligustrinum* Lodd., *Solanum tomatillo* (J.Rémy) Phil., and *Witheringia berteroana* J.Rémy. Common names include natre, natran, natren, natri, natrien, tomatilla, and tomatillo (Knapp, 1989; Muñoz *et al.,* 2004).

S. crispum is frequently used in Chilean folk medicine -including the Mapuche communities from northwestern Patagonia- in order to treat a wide variety of symptoms associated with both, acute and chronic ailments (Montes & Wilkomirsky, 1987; Muñoz *et al.,* 2004; Estomba *et al.,* 2005). It has been reported that this native plant is valuable to treat digestive diseases due to their analgesic and anti-inflammatory properties (Molares & Ladio, 2009). Infusion of leaves and stems of *S. crispum* is used to treat high blood pressure (Montes & Wilkomirsky, 1987; Muñoz *et al.,* 2004). Furthermore, infusion and enema of this plant is used to treat fever, headache, inflammation, and pain associated with sun overexposure (Santa-Cruz, 1938; Montes & Wilkomirsky, 1987; Muñoz *et al.,* 2004).

In vivo assays carried out in rabbits and guinea pigs showed that the aqueous and methanolic extracts exhibited antipyretic and anti-inflammatory activities, respectively (Delporte *et al.,* 1998). Additionally, studies evaluating the activity of the calcium release channel (CRCh) in the sarcoplasmic reticulum (SR) of porcine heart muscle incorporated into planar lipid bilayer indicate that a crude extract of *S. crispum* inactivated the CRCh (Fuentes *et al.,* 2005). According to the authors, this result may contribute to understanding the hypotensive effect associated with the use of *S. crispum* in traditional medicine. However, there is no evidence showing that *S. crispum* may directly affect vessel contractibility. Therefore, we hypothesize if *S. crispum* is used in traditional medicine, as hypotensive, then the hydroalcoholic extract of this plant will produce vasodilation of isolated rat aorta.

MATERIAL AND METHODS

Plant material and extraction

The plant was collected in Olmué, Chile in March 2012. Plant material and extracts preparation were carried at Laboratorio de Botánica, Facultad de Farmacia, Universidad de Valparaíso, Chile. The species and a voucher specimen (register Nº 1654) can be found in the Jardín Botánico Nacional Herbarium, Viña del Mar, Chile. The dried and powdered plant material containing stems and leaves y a proportion of 3:1 were macerated in ethanol-water (1:1) at room temperature for 5 days. Then the material was filtered, evaporated *in vacuo* and lyophilized, obtaining the dried hydroalcoholic extract (yield: 18.3% w/w) that was stored at 4° C. All stock solutions were freshly prepared on the experimental day. Phytochemical screening (Trease & Evans, 1989) gave positive tests for alkaloids, flavonoids, resins, saponins, and tannins.

Animals and aortic rings preparation and recording

Male Sprague-Dawley rats weighing 200-300 g were used in accordance with the United States animal use and care guidelines (NIH, 1985). The experimental protocol was approved by the Bioethical Committee of Universidad de Valparaíso (authorization number 065-2015). Rats were sacrificed by an overdose of CO_2 inhalation. Thoracic aorta was removed and mounted on a tissue chamber as previously described (Vinet *et al.,* 1991; Vinet *et al.,* 2012). Briefly, aorta was dissected, clean of connective tissue and divided into rings (4-5 mm in length). The rings were mounted gently between two L-shaped stainless steel hooks and placed in a 30 mL organ chamber containing a modified Krebs-Henseleit solution. Isometric tensions were measured using an force displacement transducer connected to a polygraph (Grass Instruments, USA). Mounted rings were tensioned to 1.5 g and allowed to equilibrate for 60 min. Endothelium function was evaluated by testing the relaxation produced by acetylcholine (1 µM) in phenylephrine (PE, 0.1 µM)-precontracted rings. Rings were repeatedly washed and allowed to equilibrate for an additional 30 min before testing the extracts. Subsequently, rings were precontracted with PE (0.1 µM) and once a stable contraction was achieved, cumulative concentration-response curves were obtained by a stepwise increase in the extract concentration (10^{-6} to 10^{-1} mg/ml). Nitro-L-arginine (L-NNA, 100 µM) was incubated with the tissue 10 min before extract addition.

Data and statistical analysis

Maximum relaxation and concentration inducing 50% of maximal relaxation (EC_{50}) was calculated using the four parameters logistic fit -also known as "4PL"- supported by GraphPad Prism 6 (GraphPad Software, Inc., USA). The relaxation from the precontracted level to the baseline was considered as 100% relaxation. Results are expressed as mean ± standard error (SE). One-way ANOVA and Tukey's multiple comparisons tests were used for statistical analysis. A P value < 0.05 was taken as statistically significant.

RESULTS AND DISCUSSION

Our results indicate that the hydroalcoholic extract of *S. crispum* has vasodilatory properties on isolated rat aorta. Figure 1 shows the concentration-response curve obtained by adding cumulative amounts of hydroalcoholic extract of *S. crispum* (10^{-6} to 1 mg/mL) on aortic rings precontracted with phenylephrine (0.1 μM). From Figure 1, we clearly note that this extract induced a concentration-dependent vasodilation of aortic rings.

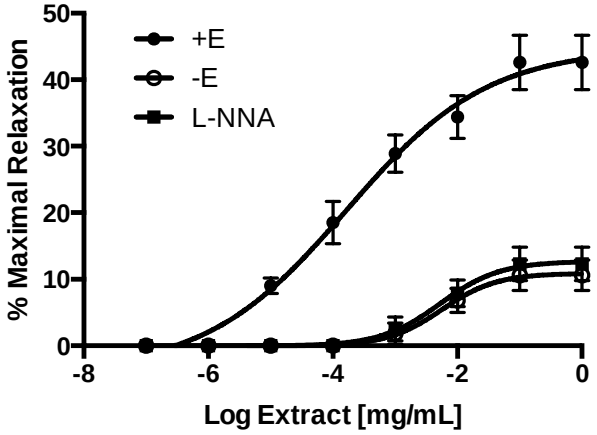

Figure 1

Concentration-response curve showing the relaxation induced by the hydroalcoholic extract of *Solanum crispum* on isolated rat aortic rings precontracted with phenylephrine (0.1 μM). +E, -E and L-NNA indicate aortic rings with endothelium, without endothelium and with endothelium preincubated with L-NNA, respectively. Concentration-response data were fitted to sigmoid curves using the four-parameter logistic function (4PL). Each data point represents mean ± SE of n = 6 experiments.

The maximal relaxation induced by the hydroalcoholic extract of *S. crispum* was significantly higher (P < 0.0001) in rings with endothelium (+E; 42.6 ± 4.1%) compared to rings without endothelium (-E; 10.6 ± 2.3%) and rings with endothelium preincubated (10 min) with nitro-L-arginine (L-NNA, 100 μM; 12.3 ± 2.5) (Figure 2). No differences were observed between rings without endothelium and those with endothelium preincubated with L-NNA (Figure 2).

Furthermore, EC_{50} values obtained for the extract tested in rings with endothelium (0.17 μg/mL) compared to rings without endothelium (5.63 μg/mL) and those pretreated with L-NNA (5.26 μg/mL) also showed significant differences (P < 0.001). No differences were observed in EC_{50} values between vessels free of endothelium and those pretreated with L-NNA. Significant differences in EC_{50} values support the notion that distinctive mechanisms are involved in endothelium-dependent and independent relaxation (Prinz, 2010). Since L-NNA is a well-known inhibitor of nitric oxide synthase (NOS) (Joly *et al.*, 1994; Mukundan & Kanagy, 2001) we conclude that vasodilation induced by the

hydroalcoholic extract of *S. crispum* is largely dependent on NO availability.

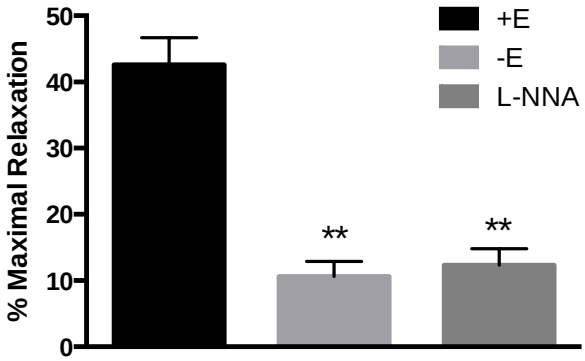

Figure 2
Maximal relaxation induced by the hydroalcoholic extract of *Solanum crispum* on isolated rat aortic rings precontracted with phenylephrine (0.1 µM). +E, -E and L-NNA indicate aortic rings with endothelium, without endothelium and with endothelium preincubated with L-NNA, respectively. ** Indicates a $P < 0.0001$ when compared to control (+E). No differences were observed between -E and L-NNA. Statistical differences were verified by one-way ANOVA and Tukey's multiple comparisons tests. Each data point represents mean ± SE of n = 6 experiments.

Our results obtained in *S. crispum* are in concordance with other studies carried out in different species of *Solanum*. Thus, aqueous and ethanolic extracts of *S. macrocarpum* significantly reduced the blood pressure in both normotensive and hypertensive rats possibly due to its diuretic activities (Iranloye *et al.*, 2011). Similarly, *S. paludosum* Moric that is commonly used to treat hypertension showed that the ethanolic extract caused hypotension and aortic vasorelaxation in rats involving both NO/sCG/PKG pathway and K^+ channels (Monteiro *et al.*, 2012). In contrast, aqueous extracts of *S. rostratum* Dunal induced a concentration-dependent contraction of rat aorta rings, suggesting that this plant could be beneficial for the treatment of venous insufficiency (Ibarra-Alvarado *et al.*, 2010).

In conclusion, the vasodilatory effect induced by the hydroalcoholic extract of *S. crispum* in isolated precontracted rat aortic rings was mainly endothelium-dependent and mediated by NO. Further complementary studies on this extract and *in vivo* studies are needed to continue the search for potential antihypertensive agents present in this plant.

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