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Isolation of hypotensive compounds from *Solanum sisymbriifolium* Lam.

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Abstract

The crude hydroalcoholic root extract (CRE) of *Solanum sisymbriifolium* Lam. has formerly been shown to have hypotensive activity both in normo-and hypertensive rats. Hypotensive activity-guided fractionation of the CRE was performed in anaesthetized normotensive rats, which led to the isolation of the active principles. The intravenous (i.v.) and intraperitoneal (i.p.) values of the CRE in mice were found to be, respectively, 343 and 451 mg/kg, and no lethal effect was caused by doses up to 5.0 g/kg when administered by oral route. Depression of locomotion, increase of breathing rate and piloerection was observed in a general behavior test with doses up to 200 mg/kg i.p., and 1000 mg/kg p.o., respectively. Increase in the gastrointestinal transit was found using 0.1 g/kg, whereas at doses of 0.5 and 1 g/kg, no significant activity was observed in comparison with the control mice. Hexanic and butanolic fractions induced a remarkable hypotension in anaesthetized normotensive rats in doses of 1, 5, 7.5 and 10 mg/kg i.v. Two compounds isolated from the butanolic fraction induced a significant decrease of the blood pressure, HR, amplitude of the ECG and breathing rate when injected in a dose of 1 mg/kg i.v; and both systofic and diastolic, blood pressures were affected in a proportional mode. The hypotensive effect of the two compounds were not influenced by pretreatment with atropine and propranolol; and the pressor response to noradrenaline was not affected by any of them which suggests that neither a direct muscarinic activity, β-adrenoceptor activation nor decrease of sympathetic vascular tone (sympatholitic activity) are probably involved in the mechanism of hypotension. The present study shows that the CRE of *S. sisymbriifolium* contains at least two hypotensive compounds whose characterization is under way.

Keywords: *Solanum sisymbriifolium*; Acute toxicity; Gastrointestinal transit; General behavior; Hypotensive activity; Isolation

1. Introduction

Most people in developing countries have poor access to modern health care, including drugs used for the treatment of different diseases (Waller,

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1993). In Paraguay, traditional practitioners are abundant who prepare ethnomedicine, some of which are clearly simple placebos. Therefore, pharmacological validation of medicinal plants or ethnomedical treatment methods could greatly benefit populations with poor economic resources.

The root of *Solanum sisymbriifolium* Lam. (Solanaceae) (ñuatí pytâ), a perennial herb found in eastern Paraguay, is used as diuretic and antihypertensive in the Paraguayan folk medicine (Gonzales Torrez, 1992). The crude hydroalcoholic root extract (CRE) of *S. sisymbriifolium* has been found to possess a strong hypotensive effect (Ibarrola et al., 1996), both by oral and intravenous administration, in adrenal regeneration hypertensive (ARH) rats.

The present paper reports, first of all, studies on the acute toxicity, effect on general behavior and gastrointestinal transit of the CRE as a measure of the potential activities on both central and autonomous nervous systems. On the other hand, a hypotensive activity-guided fractionation of the extract was performed on anaesthetized non-notensive rats. Purification of the most active fraction was finally performed in order to identify the active(s) hypotensive principle(s).

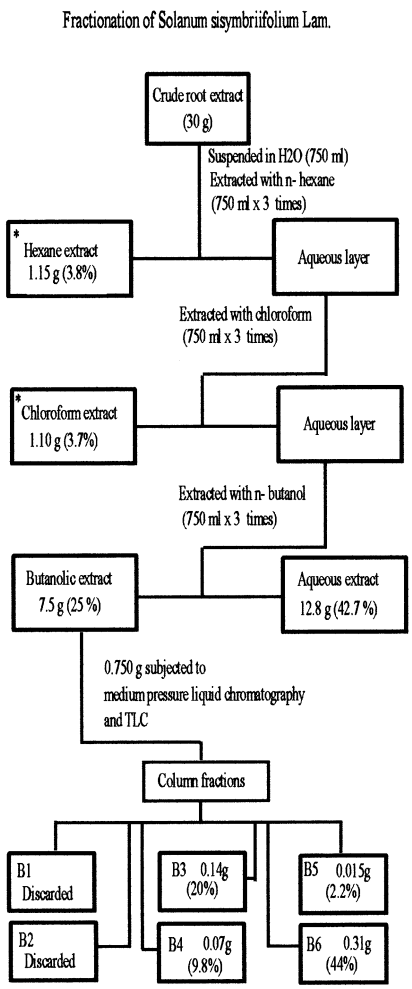

Fig. 1. Flow diagram of fractionation of the crude root extract from *S. sisymbriifolium* Lam. Number in parenthesis indicate percent yields of each fraction (*dried with sodium sulphate anhydrous).

2. Materials and methods

2.1. Extraction and fractionation

The CRE of *S. sysimbriifolium* Lam. was prepared from 3 kg of fresh roots collected in the Eastern Region of Paraguay and identified at the Herbarium of the Faculty of Chemical Sciences, where voucher specimens have been deposited (Soria 5248). The roots were air-dried at room temperature and reduced to fine powder by milling. The resulting powder (1335 g) was subjected to reflux extraction with 6.5 l of an ethanol-water (7:3) mixture, three times, 1 h each. The combined extracts were filtered and concentrated under reduced pressure and then freeze-dried at 20°C and 60 mTorr (Nihon Freezer FP 1.5, Sanko Irika), yielding 126 g of CRE. Thirty grams of CRE were suspended in 750 ml of deionized water and extracted with hexane, chloroform and n-butanol, using 750 ml of each solvent three times. Hexane and chloroform extracts were dried with anhydrous sodium sulphate and evaporated under reduced pressure. Butanol extract was concentrated under reduced pressure and the water fraction was freeze-dried (see Fig. 1). A 0.704-g aliquot of the butanol fraction was subjected to medium pressure liquid chromatography in a column filled with 31 g of TLC-grade silica gel without binder (Aldrich), using chloroform-methanol mixtures delivered by a medium pressure liquid pump (FMI Lab Pump RP-SY, Fluid

Metering), as eluent. The polarity of the mobile phase was changed from 95:5 to 70:30 (chloroform: methanol), and 110 fractions of 10 ml each were collected- The resulting fractions were subjected to analytical TLC on silica gel-precoated plastic plates (Schleilcher & Schüll GmbH), eluted with chloroform-methanol mixtures and developed by spraying with a mixture of acetic acid-water-sulphuric acid (80:16:4) and heating at 100°C, and Dragendorff reagent. According to the TLC results, the fractions from the column were clustered in six groups (B1: tubes 1–30, B2: tubes 31–82, B3: tubes 83–88, B4: tubes 89–93, B5: tubes 94–96, and B6: tubes 97–110).

2.2. Animals

Swiss adult albino mice of either sex weighing between 20 and 30 g were used to study the LD50 by both i.p. and oral administration, the general behavior activity and the effect on gastrointestinal transit (propulsion) of the CRE. Albino normotensive rats (Wistar strain) of either sex, weighing 250–350 g, were used for the study of the effect of the CRE and its different fractions on the blood pressure of anaesthetized rats. A 12-h dark-light cycle, 23–25°C temperature, and 50–60% humidity was maintained inside the animal room. The animals received a standard food and, prior to experimentation, were fasted overnight with access to water ad libitum.

2.3. Drugs

Heparin, atropine, neostigmine and sodium chloride were obtained from Sigma Chemical Company (St Louis, MO, USA); pentobarbital (Nembutal) from Abbott (Japan), n-hexane, n-butanol and chloroform were obtained from Merck; propylenglycol and charcoal for pharmaceutical use were purchased locally.

2.4. Acute toxicity (LD50) and effect on gross behavior

The extract was suspended in saline containing 1% propylenglycol and administered: (a) intravenously (b) intraperitoneally and (c) orally, to groups of ten mice. After 24 h, the death-toll, in percentage, was calculated and converted into probit units. The LD50 was calculated from a plot of the probit units versus logarithm of the dose according to the method described previously (Staff of the Department of Pharmacology, University of Edinburgh, 1970). The effect on spontaneous behavior of the mice was performed using the hippocratic procedure (Malone, 1977). Groups of five adult albino mice were administered: (A) vehicle, 10, 30, 100 and 200 mg/kg i.p. and B) vehicle, 100, 300, 500 and 1000 mg/kg p.o. of CRE suspended in 1% propylenglycol used as a control. The mice were kept under observation for 2 weeks.

2.5. Gastrointestinal transit in mice

Groups of ten mice were treated with: (A) 0.3 μl p.o. of distilled water, (B) 10 g/kg s.c. of neostigmine methyl sulphate, (C) 1 mg/kg i.p. of atropine sulphate, (D), (E) and (F) with 0. 1, 0.5 and 1.0 g/kg p.o. of CRE suspended in saline. Both chemicals and extract were administered in a volume of 0.1 ml/10 g body weight. Thirty minutes later, 0.3 ml of charcoal (10% aqueous suspension) was administered to all animals by oral route. After 40 min, the mice were sacrificed by ether anaesthesia and cervical dislocation; the small intestine was rapidly and carefully removed and aligned parallel to a ruler. The length traversed by the charcoal was calculated as a percentage of the total intestine length (from pyloric sphincter to ileocecal junction).

2.6. Intact preparation of anaesthetized normotensive rats

Wistar rats of either sex (150–300 g) were anaesthetized with pentobarbital (35 mg/kg, i.p.) and cannulations were performed as described previously (Staff of the Department of Pharmacology, University of Edinburgh, 1970). The arterial blood pressure was recorded from the carotid artery with a Gould Statham P23 ID transducer connected to a blood pressure amplifier unit, model 1257 (Nec-San Ei Instruments). The femoral vein was cannulated for intravenous ad-

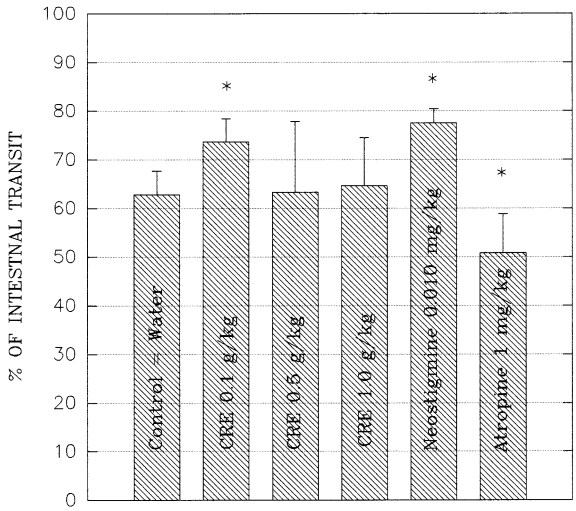

Fig. 2. Effect of increasing doses of CRE (0.1, 0.5 and 1.0 g/kg, p.o.) of *S. sisymbriifolium* Lam., vehicle (control = water), neostigmine and atropine on intestinal transit in mice. Each bar represents the mean ± SD and $*P < 0.05$ were considered significant.

ministration of the drugs. Electrocardiogram (ECG) was recorded by attaching needle electrodes inserted through the skin into the muscle and connected to a bioelectric amplifier unit model 1253A (Nec-San Ei Instruments). Heart rate (HR) was measured with a tachometer model 1321 (Nec-San Ei Instruments) connected to the output of an ECG bioelectric amplifier. The trachea was cannulated and the breathing rate was measured. The exhaled breath was analyzed using a CO_2 Monitor Respina 1H31 (Nec-San Ei Instruments). Hexanic, chloroformic and butanolic fractions were dissolved in a mixture of 10% ethanol, 40% propylenglycol and destfled water. Intravenous administration (0.1 ml/100 g body weight) of this vehicle, prior to the samples, were used as a control.

2.7. Statistical analysis

The results are expressed as mean ± SD and the statistical analysis of the data was performed by the Student's *t*-test. Probability level of < 0.05 was considered as statistically significant.

3. Results

3.1. Acute toxicity and effect on general behavior

The intravenous and intraperitoneal LD50 were found to be 343 and 451 mg/kg, respectively, in 24 h of observation (95% CI: 330–360 mg/kg i.v.; 293–704 mg/kg i.p.). Oral administration of doses up to 5.0 g/kg did not provoke any toxic symptoms in the mice. Concerning the effect on general behavior, the CRE elicited a rapid-onset, dose-related decrease of the spontaneous motility, increase of the breathing rate and piloerection within 5–10 min of i.p. (10, 30, 100 and 200 mg/kg) and 40–50 min of p.o. (100, 300, 500 and 1000 mg/kg) administration to the animals. Doses superior to 5.0 g/kg p.o. were not used because they yield no creditable parameter (CEME, 1982).

3.2. Effect on gastrointestinal transit

CRE at a dose of 0.1 g/kg p.o. significantly increased the intestinal transit in comparison with the control. However, it had no effect at doses of 0.5 and 1.0 g/kg (Fig. 2).

3.3. Effect of CRE and fractions on the blood pressure of anaesthetized nortnotensive rats

Four different doses (1, 5, 7.5 and 10 mg/kg i.v.) of aqueous and chloroformic fractions administered, respectively, to anaesthetized normotensive rats did not produce a significant decrease in blood pressure, HR, ECG and respiration rate. However, a slight and not significant increase in blood pressure was observed with the chloroformic fraction.

A clear hypotensive effect was observed on anaesthetized normotensive rats when the hexanic fraction was administered intravenously at doses of 1, 5, 7.5 and 10 mg/kg (Fig. 3A). Likewise, the administration of the butanolic fraction in anaesthetized normotensive rats at doses of 1, 5, 7.5 and 10 mg/kg, i.v. induced a dose-dependent decrease in arterial blood pressure (Fig. 3B). Bradycardia, decrease of the ECG amplitude and bradypnea were induced, in a dose dependent fashion, by both hexanic and butanofic fractions.

The purified subfractions of the butanolic extract, called B3 and B5, respectively, induced a significant decrease in the blood pressure, when injected in a dose of 1 mg/kg i.v. Both the systolic and diastolic blood pressure appeared to be affected in a proportional mode (Fig. 4). A decrease in the HR ($28.37 \pm 8.1\%$), decrease in the amplitude of the ECG ($10.2 \pm 5.8\%$), the decrease of the breathing frequency ($8.3 \pm 2.1\%$) and the amplitude of the exhaled breath ($13.48 \pm 2.2\%$) were observed upon administration of B3 (1 mg/kg i.v.). Similarly, intravenous administration of B5 (1 mg/kg) induced a decrease in the HR ($27.28 \pm 4.2\%$), decrease in the amplitude of the ECG ($34.1 \pm 7.2\%$), the decrease in the breathing frequency ($25.1 \pm 5.2\%$) and the amplitude of the exhaled breath ($20.3 \pm 8.2\%$). The hypotensive effect of the butanolic fraction (5 mg/kg i.v.), and the B3 (1 mg/kg i.v.) and B5 (1 mg/kg i.v.) subfractions were not influenced by previous (5 min) injection of propranolol (1 mg/kg i.v.) and atropine (1 mg/kg i.v.), respectively. The pressure response to noradrenaline (1 μg/kg i.v.) was not affected by pretreatment with the butanolic fraction (5 mg/kg i.v.), and the B3 (1 mg/kg i.v.) and B5 (1 mg/kg i.v.) subfraction, respectively. Subfractions B1 and B2 were of minor components as shown by TLC analysis and were discarded. Finally, B4 and B6 did not affect the blood pressure of anaesthetized normotensive rats when injected in a dose of 1 mg/kg i. v.

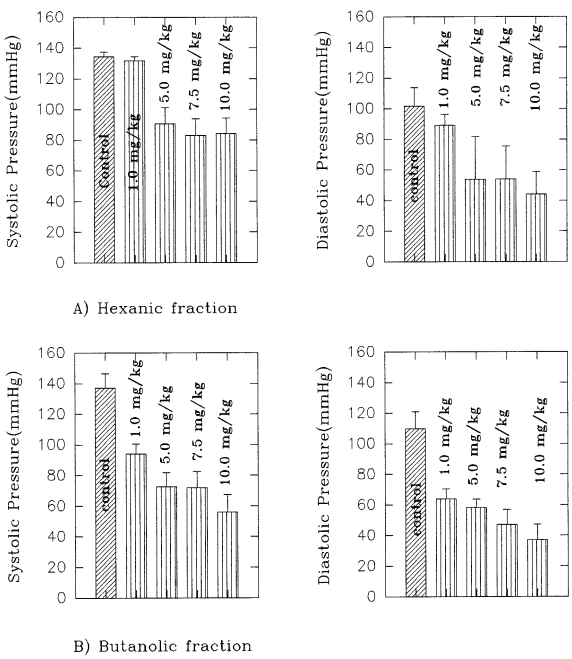

Fig. 3. Systolic and diastofic blood pressure variation in anaesthetized normotensive rats after intravenous administration of (A) hexanic and (B) butanolic fractions of *S. sisymbrifolium* Lam., in doses of 1.0, 5.0, 7.5, and 10.0 mg/kg, respectively. Five animals were used for each dose and each bar represents the mean ± SD $*P < 0.05$ was considered as significant.

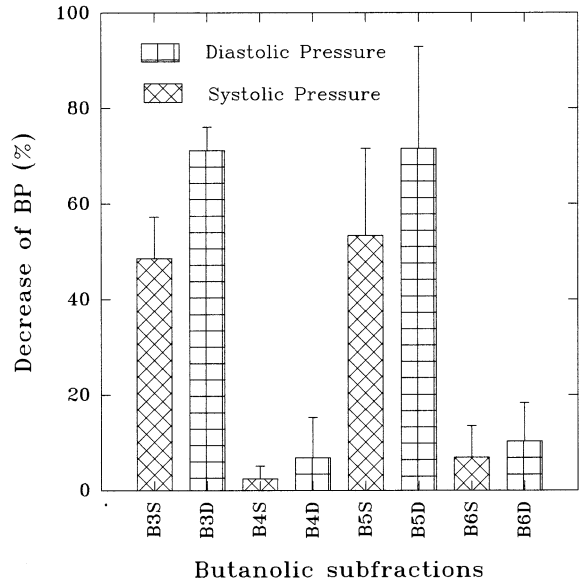

Fig. 4. Effect of intravenous administration of 1.0 mg/kg of four butanolic subfractions (B3, B4, B5 and B6) of *S. sisymbriifolium* Lam. on systolic and diastolic, blood pressure in anaesthetized normotensive rats (S = systobc. D = diastolic). Each bar represents the mean ± SD and $*P < 0.05$ were considered significant.

3.4. Chromatographic characterization of the fractions

The major hypotensive compounds were found in the subfractions B3 and B5. None of them yielded positive Dragendorff reaction for alkaloids. B3 to B6 showed brown spots on TLC plates after spraying with acetic acid-water-sulphuric acid (80:16:4) followed by heating at 100°C.

Fractions B1 and B2 showed only low amount of components, were < 5 mg each, just visible on UV illumination (254/360 nm). The TLC behaviors, of the rest of the fractions, on silicagel, developed with the mixture chloroform-acetic acid-methanol-water (6:3:1:0.5) were as follows: B3 (0.14 g) consisting of a single spot ($Rf = 0.78$), B4 (0.07 g), the major one, showing two spots with $Rf = 0.42$, and traces were present in the B3 fraction. The fraction B5 (0.015 g) showed three spots, those observed in the B4 fraction and an additional spot with intermediate *Rf* value (0.56), the major one having an *Rf* value of 0.42. Finally, the B6 fraction (0.31 g) appeared to be a mixture of two groups of components, the minor one composed of the formerly described spots (B3, B4 and the additional compound which appears in B5) and other three spots of higher polarity with *Rf* values of 0.26, 0.20 and 0. 11, respectively.

4. Discussion and conclusions

First of all, the present study demonstrates the low acute toxicity of the crude hydroalcoholic root extract (CRE) of *S. sisymbriifolium* Lam. (ñuati-pytâ) when administered by intravenous, intraperitoneal and oral route. No behavioral changes were observed in the general pharmacological test. However, more specific assays (spontaneous motility, motor co-ordination, respiration, sedative or hypnotic activity) will be needed to assess the detailed activity of the extract on the central nervous system. The activity on gastrointestinal transit was not dose-related and, interestingly, increased propulsion induced by low doses while a decrease at higher doses was observed.

In a previous paper we had reported the hypotensive properties of the CRE of *S. sisymbriifolium* when administered to ARH hypertensive rats (Ibarrola et al., 1996). Herein, in a new series of experiments on blood pressure of anaesthetized normotensive rats, we have found the hypotensive activity in the hexanic and butanolic fractions. The CRE of *S. sisymbriifolium* yielded ≈ 25 and 3.8% of butanolic and hexanic portions (lyophilized), respectively, both of them showing a very strong hypotensive activity. Purification of the butanolic fraction through medium pressure chromatography and TLC was decided because of the high yield and the activity observed in this fraction. From the resulting subfractionation, which yielded six groups, B5 induced a significant decrease in blood pressure, and B3 appeared to contain the most potent hypotensive principle.

The hypotensive effect of B3 and B5 were not influenced by pretreatment with atropine and propranolol. This indicate that the effect of B3 and B5 are probably no mediated neither by direct muscarinic and nor β adrenergic activation, respectively. On the other hand, the pressor response to noradrenaline was affected by neither B3 nor B5 showing a low capacity to decrease the sympathetic vascular tone.

Finally, the use of *S. sisymbriifolium* (ñuati-pytâ) in folk medicine has a nice correlation with scientific data, since two of the compounds isolated have shown significant hipotensive activity in rats. Further studies to investigate the mechanism(s) of action and the structural determination of the active principle present in B3 and B5 are now in progress.

Acknowledgements

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