Pharmacological Evidence of α_2 -Adrenergic Receptors in the Hypotensive Effect of *Platonia insignis* Mart.

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ABSTRACT *Platonia insignis* Mart. (Clusiaceae) is a medicinal plant from the Brazilian Amazon region. The present study evaluated the biological potential of the ethanol extract (*Pi*-EtOH) and ethyl acetate fraction (*Pi*-EtOAc) of the *P. insignis* fruit shells on the cardiovascular system of rats. *Pi*-EtOH or *Pi*-EtOAc (12.5, 25, and 50 mg/kg) was administered intravenously in normotensive rats (260–300 g), and the mean arterial pressure and the heart rate were monitored. The *Pi*-EtOH induced hypotension (-11.56 ± 0.89 , -7.43 ± 0.85 , and -17.56 ± 1.97 mmHg) followed by bradycardia in two highest doses (-8.89 ± 3.62 and -15.79 ± 1.83 beats/min) and *Pi*-EtOAc, at the same doses, induced hypotension (-11.2 ± 1.03 , -14.48 ± 1.13 , -29.89 ± 2.67 mmHg) more intensively, followed by tachycardia at the dose 12.5 and 25 mg/kg (15.64 ± 2.06 , 19.31 ± 1.92 beats/min) and bradycardia at a dose of 50 mg/kg (-9.98 ± 7.33 beats/min). The hypotensive response from *Pi*-EtOAc was not attenuated when used in the pretreatment with L-NAME, verapamil, propranolol, and hexamethonium. However, when using yohimbine, the hypotensive effect was inhibited (-4.42 ± 1.28 ($P < .05$), -3.29 ± 0.99 ($P < .05$), 2.06 ± 1.18 mmHg ($P < .05$); Student's *t*-test). Hence, the *Pi*-EtOAc seems to act similarly to the α_2 -adrenergic agonist in this hypotensive effect.

KEY WORDS: • blood pressure • cardiovascular • hypotension • *Platonia insignis* • yohimbine

INTRODUCTION

HYPERTENSION is one of the most common and rapidly spreading cardiovascular diseases and is a major cause of morbidity and mortality in mankind, as well as an important risk factor for the development of other cardiovascular diseases.¹ The conventional therapy for hypertension is not always safe, efficacious, and it is also beyond therapeutic measures.² Phytotherapy is the most accessible and/or affordable option for a large proportion of the world's population, which looks for an alternative popular remedy. In addition, a number of traditional systems of medicine are heavily based on the use of herbs as medicine. At the same time, there is a global revival of interest in the use of botanicals, and physicians of the modern medicine are now beginning to accept the traditional remedies following scientific validation.³

An important alternative is the bacuri fruit (*Platonia insignis* Mart., Clusiaceae), a native species from the Brazilian Amazon region with a wide range of applications, primarily

found in the state of Piauí, Maranhão, and Pará (Brazil). It is composed by pulp, seeds, and shell, and its fruit pulp is widely used as a flavoring agent in the preparing of ice creams, jams, and yogurts. In folk medicine, the decoction of seeds is used to treat diarrhea, while the seed oil is used both against spider or snake bites and also in the treatment of skin problems, otitis, rheumatism, and arthritis.⁴ Besides, pharmacological studies have demonstrated that the ethyl acetate fraction from *P. insignis* seeds possesses an anticonvulsant potential on pilocarpine-induced seizures in rats.⁵

The *P. insignis* fruit shells are characterized as a product with no exploitation. However, some secondary metabolites were identified as α -terpineol and β -bisabolene, and the isolated constituents were predominantly free fatty acids: palmitic, oleic, linoleic, α -linolenic, and stearic acids. In addition to these fatty acids, the LCO₂-EtOH extracts contained caprylic and myristic acids, alcohols (linalool and 3,7-dimethyloct-1-en-3,7-diol), and the phenol ether eugenol, which remarkably increased its value due to the great source of compounds bearing several biological properties.⁶

Considering some reported application for *P. insignis* fruit shells, the present research was carried out to evaluate the biological potential of *P. insignis* fruit shells on the cardiovascular system of normotensive rats, as well as to

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investigate the possible mechanisms of action underlying these pharmacological effects.

MATERIALS AND METHODS

Preparation of extracts and fractions from the Platonía fruit shells

Plant material and *P. insignis* shells were obtained in the state of Maranhão in 2012 and then identified by a botanist (Profa. Dra. Roseli Farias de Melo Barros). The voucher specimen (TEPB 20701) was deposited in the Graziela Barroso Herbarium of the Federal University of Piauí. Fruit shells were cleaned, cut into small pieces of ~1 cm, and then lyophilized. The powdered material (245.5 g) was soaked in 95% ethanol solution in a large container for 5 days with occasional shaking.⁷ This procedure was repeated for over six times and the combined filtrate was evaporated on a rotary evaporator, under reduced pressure to a thick semi-solid mass of yellowish color, that is, the crude extract of *P. insignis* (*Pi*-EtOH, 79.9 g), yielding 35%. The 79.9 g of *Pi*-EtOH was suspended in 700 mL of MeOH/H₂O (1:1) and subjected to liquid–liquid partition with hexane and ethyl acetate. The mixture of MeOH/water (1:1) was first subjected to liquid–liquid extraction for four times with 200 mL of hexane. The hexane phase was separated from the hydromethanol phase by means of a decanting process and then was concentrated on a rotary evaporator under reduced pressure and lyophilized to yield 18.4 g (23.2%) of hexane fraction. Subsequently, the hydromethanol fraction was subjected to liquid–liquid extraction for four times with 350 mL of ethyl acetate. In the EtOAc phase, with intermediate polarity, hydromethanol was separated by means of a decanting process. During this separation process, there was the formation of a precipitate in the EtOAc phase, which was separated by simple filtration, dried in a rotary evaporator under reduced pressure and lyophilized, resulting in 33.0 g (41.7%) ethyl acetate fraction from bacuri shell fruits (*Pi*-EtOAc). The remaining aqueous phase obtained after the extraction with EtOAc yielded 18.0 g (22.7%) after lyophilization. The *Pi*-EtOH samples and *Pi*-EtOAc were frozen in liquid nitrogen (approximately –190°C) and the residual water was removed in lyophilizer Micro Modulyo Edwards coupled to a high-performance valPump VLP80 Savant vacuum pump (–50°C, 5×10^{-2} mbar).

Animals

Male Wistar normotensive rats (200–300 g) were obtained from the Animal Care Facilities of the Center for Research on Medicinal Plants, Federal University of Piauí, Brazil. They were kept in a large cage under controlled conditions of temperature and lighting (lights on: 06:00–18:00 h), fed on a rodent diet, and *ad libitum* tap water. All procedures were approved by the ethics committee on animal experimentation of the Federal University of Piauí, Brazil (Protocol number N°78/10) and were in compliance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication 85–23, revised 1996).

Drugs and reagents

The drugs used were N^o-nitro-L-arginine methyl ester hydrochloride, cremophor, sodium nitroprusside, verapamil, prazosin, yohimbine, propranolol, hexamethonium (all from Sigma, St. Louis, MO, USA), sodium thiopental (Cristalia, São Paulo, Brazil), and heparin (Ariston, São Paulo, Brazil). In the preparation of the stock solutions, either *Pi*-EtOH or *Pi*-EtOAc was diluted in deionized water/cremophor (0.5% v/v) solution, for *in vitro* experiments, or saline/cremophor (0.5% v/v) solution, for *in vivo* experiments. Other drugs were diluted in saline (*in vivo*) or deionized water (*in vitro*) solutions only. All stock solutions were maintained at 0°C and diluted to the desired concentration with deionized water or saline, when necessary. Dimethyl sulfoxide (DMSO) in the concentrations used showed no effect in control experiments.

Effect of Pi-EtOH or Pi-EtOAc on hemodynamic parameters in nonanesthetized rats

The measurements of the mean arterial pressure (MAP) and heart rate (HR) were performed as described by Oliveira *et al.*⁸ Sodium nitroprusside (10 µg/kg; i.v.) was injected to check the efficacy of the venous catheter insertion. After the stabilization period, MAP and HR were recorded in conscious and free-moving rats before (baseline values) and after i.v. *in bolus* administration of *Pi*-EtOH or *Pi*-EtOAc (12.5, 25, and 50 mg/kg) for the obtainment of dose–response curves. Successive injections of *Pi*-EtOH or *Pi*-EtOAc were well separated by a time interval sufficient to allow full recovery of the basal parameter (30 min). To elucidate the possible mechanisms underlying the hypotensive response, *Pi*-EtOAc was administered to animals pretreated with one of the following drugs: a nitric oxide synthase (NOS) competitive inhibitor (L-NAME, 20 mg/kg),⁹ a ganglionic blocking agent (hexamethonium, 20 mg/kg),¹⁰ a β-adrenergic blocking agent (propranolol, 20 mg/kg),¹¹ an α-adrenergic blocking agent (yohimbine, 2.5 mg/kg),¹² or a calcium channel block (verapamil, 20 mg/kg).¹³ After protocols, all animals were killed with sodic thiopental anesthesia (100 mg/kg; i.p.).

Statistical analysis

Results are expressed as the mean values ± standard error of means of four to eight animals. The statistical evaluation was carried out using the unpaired Student's *t*-test). In all analyses, a *P*-value < .05 was considered as statistically significant. All procedures were performed by using Graph Pad Prism 5.0™ (Graph Pad Software, Inc., San Diego, CA, USA).

RESULTS

Effect of Pi-EtOH or Pi-EtOAc on MAP and HR in nonanesthetized rats

In this study, the baseline values of MAP and HR were 112 ± 6 mmHg and 350 ± 13 bpm, respectively ($n = 5$). After stabilization, the administration of *Pi*-EtOH or *Pi*-EtOAc at doses of 12.5, 25, and 50 mg/kg i.v. induced hypotension by 11.6 ± 0.9 , -7.4 ± 0.85 , -17.6 ± 2.0 and -11.2 ± 1.0 , -14.5 ± 1.1 , -30.0 ± 2.7 mmHg, respectively (Fig. 1A).

FIG. 1. *Pi-EtOH*- and *Pi-EtOAc*-induced cardiovascular effects on mean arterial pressure (MAP) (mmHg) (**A**) and heart rate (HR) (bpm) (**B**) with their respective original traces (**C**, **D**) in nonanesthetized normotensive rats ($n=5$). Values are expressed as mean $\pm$ standard error of means (SEM). ** $P<.01$, *** $P<.001$ versus *Pi-EtOH* (nonpaired Student's t -test).

This response was accompanied by the bradycardic effect in doses (25 and 50 mg/kg) for *Pi-EtOH* (-8.9 ± 3.6 and -15.8 ± 1.8 beats/min, respectively), while in fraction *Pi-EtOAc*, we observed the tachycardic effect (15.6 ± 2.0 and 19.3 ± 1.9 beats/min in doses 12.5 and 25 mg/kg, respectively) and bradycardia at the highest dose (-10.0 ± 7.3 beats/min) (Fig. 1B). The duration of *Pi-EtOH* or *Pi-EtOAc* effect on MAP and HR was ~ 05 sec. The original traces demonstrate a better hypotensive effect for the *Pi-EtOAc*. Therefore, the following experiments were performed with this fraction (Fig. 1C, D).

Effect of L-NAME or hexamethonium on *Pi-EtOAc*-induced responses in nonanesthetized rats

Administration of L-NAME significantly potentiated the hypotensive response in doses [-21.5 ± 1.8 ($P<.05$); 50.4 ± 2.4 mmHg ($P<.001$), respectively vs. *Pi-EtOAc*], but did not significantly attenuate the tachycardic response (Fig. 2A–C). A similar response was obtained with the administration of hexamethonium at doses 12.5 and 25 mg/kg [-21.8 ± 1.09 ($P<.001$), -21.9 ± 1.0 mmHg ($P<.001$), respectively; Student's t -test] and significantly attenuated a tachycardic response (Fig. 3A–C).

Effect of verapamil or propranolol on *Pi-EtOAc*-induced responses in nonanesthetized rats

Pretreatment with verapamil did not alter the cardiovascular effect induced by EtOAc (-17.5 ± 3.0 , -23.5 ± 6.9 ,

-40.6 ± 5.5 mmHg and 15.4 ± 5.8 , 4.4 ± 8.7 , -5.0 ± 4.8 beats/min, respectively) (Fig. 4A–C). In the presence of propranolol, there was no potentiation in the amounts of MAP, only at a dose of 25 mg/kg [-36.4 ± 6.9 mmHg ($P<.01$) vs. 25 mg/kg *Pi-EtOAc*], with little interference in cardiac frequency in (12.5 and 25 mg/kg) doses (Fig. 5A–C).

Effect of yohimbine on *Pi-EtOAc*-induced responses in nonanesthetized rats

The pretreatment with yohimbine completely abolished the *Pi-EtOAc*-induced hypotensive and tachycardic responses at doses of 12.5 and 25 mg/kg [-4.4 ± 1.2 ($P<.01$), -3.3 ± 0.9 mmHg ($P<.001$), vs. *Pi-EtOAc*] and promoted reversion of hypotension at a dose of 50 mg/kg [2.05 ± 1.2 mmHg ($P<.001$) vs. *Pi-EtOAc*]. The values obtained were not different from values of MAP with saline nor were there changes in HR (Fig. 6A–C).

DISCUSSION

This study showed that the ethanol extract (*Pi-EtOH*) and the ethyl acetate fraction (*Pi-EtOAc*) obtained from the *P. insignis* Mart. fruit peel promoted a powerful hypotensive effect, where the semipurified fraction presented a stronger hypotensive effect acting on the α -adrenergic receptors. The (i.v.) administration of *Pi-EtOH* in normotensive rats induced the hypotension followed by bradycardia in a fast and mild way (latency time of 3–5 sec) and the *Pi-EtOAc* in the two smaller doses promoted hypotension with a higher

FIG. 2. Original traces of the *Pi*-EtOAc-induced hypotensive effect (A) and on MAP (mmHg) (B) and HR (bpm) (C) after acute treatment with L-NAME (20 mg/kg, i.v.; $n=4$) in nonanesthetized normotensive rats. Values are expressed as mean $\pm$ SEM. * $P < .05$, *** $P < .001$ versus *Pi*-EtOAc (nonpaired Student's t -test).

intensity followed by a tachycardic response, while the 50 mg/kg dose induced hypotension followed by bradycardia. All those effects are reversible, eliminating then the possibility of their being related to toxic effects of EtOH-*Pi* and *Pi*-EtOAc. The results obtained showed that both EtOH-*Pi* and *Pi*-EtOAc presented hypotensive activities. However, the fraction presented more efficacy in causing hypotension in relation to the extract. Due to that, we started investigating the mechanism of action only with *Pi*-EtOAc.

The release of large amounts of nitric oxide (NO) by the NOS may inhibit the induction of COX-2 and suppress the formation of metabolites of the COX.^{14,15} Because of that, the treatment with L-NAME in rats causes lesions in the vascular endothelium, promoting the unbalance of these modulatory actions. The reaction of NO with the superoxide

FIG. 3. Original traces of the *Pi*-EtOAc-induced hypotensive effect (A) and on MAP (mmHg) (B) and HR (bpm) (C) after acute treatment with hexamethonium (20 mg/kg, i.v.; $n=4$) in nonanesthetized normotensive rats. Values are expressed as mean $\pm$ SEM. ** $P < .01$, *** $P < .001$ versus *Pi*-EtOAc (nonpaired Student's t -test).

radical causes the formation of peroxynitrite, which is highly oxidative and nitrating, damaging the endothelium and resulting in an acute status of arterial hypertension.^{15,16} When the effect of the NO restriction is investigated, L-NAME is used both in acute doses and in chronic doses *in vivo*.¹⁷ Aiming at evaluating whether NO could possibly be playing a role in the hypotensive effect, caused by the fraction studied, the rats were pretreated with L-NAME 30 min before the administration of the fraction doses. It was observed that the effect of the *Pi*-EtOAc was neither inhibited nor attenuated with the NOS blockade, suggesting that the hypotensive effect in the presence of L-NAME does not depend on the NO synthesis; that hypotensive action caused by the fraction excludes the participation of both NO and the L-arginine-NO-GMPc pathway.

To evaluate whether the effect of *Pi*-EtOAc could be related to the blockade of the Ca^{2+} channels, we performed

FIG. 4. Original traces of the *Pi*-EtOAc-induced hypotensive effect (A) and on MAP (mmHg) (B) and HR (bpm) (C) after acute treatment with verapamil (1.0 mg/kg, i.v., $n=4$) in nonanesthetized normotensive rats. Values are expressed as mean $\pm$ SEM.

a pretreatment with verapamil: a blocker of the L-type Calcium channels, which induces the generalized systemic vasodilatation, consequently reducing the vascular resistance causing a drop in the arterial pressure and inducing also depression in the electrical conduction in sinus and atrio-ventricular nodes, causing bradycardia.^{18–20} The endovenous administration of verapamil reaches maximum effect in 15 min and after its application has a half-life from 90 min to 5 h.²¹ The administration of *Pi*-EtOAc after verapamil showed that there was no significant alteration of the MAP and HR, thus suggesting that the *Pi*-EtOAc promotes hypotension without the participation of the blockade of Ca^{2+} channels.

We targeted the assessment of the *Pi*-EtOAc influence over the β_1 -adrenergic receptors, which are located in a higher concentration in the heart, whereas the β_2 -adrenergic receptors are primarily in the smooth muscle (vascular and

FIG. 5. Original traces of the *Pi*-EtOAc-induced hypotensive effect (A) and on MAP (mmHg) (B) and HR (bpm) (C) after acute treatment with propranolol (1.5 mg/kg, i.v.; $n=4$) in nonanesthetized normotensive rats. Values are expressed as mean $\pm$ SEM. ** $P < .01$ versus *Pi*-EtOAc (nonpaired Student's *t*-test).

bronchi). Such receptors are coupled to the G_s protein, bound to the adenylate cyclase and, when activated by antagonists (catecholamines), increase the cyclic adenosine monophosphate (cAMP), thus activating the protein kinase A (PKA), which phosphorylates the Ca^{2+} channels increasing the $[\text{Ca}^{2+}]_i$. Due to that, there is an increase in the fractional shortening of the cardiac muscle during contraction (positive inotropic effect) and a speedup in the HR (negative chronotropic effect), causing the increase of the cardiac output.²² Likewise, we ran experiments using propranolol as a pharmacological tool, which is a nonselective β -adrenergic antagonist.^{11,23} In the presence of the *Pi*-EtOAc fraction, we observed that the blockade of the β -adrenergic receptors did not cause a decrease in the hypotensive effect; instead of that, there was a significant increase of the hypotensive tension in an intermediate dose, showing that the hypotensive effect of

FIG. 6. Original traces of the *Pi*-EtOAc-induced hypotensive effect (A) and on MAP (mmHg) (B) and HR (bpm) (C) after acute blockade of α_2 -adrenergic receptors (yohimbine 2.0 mg/kg, i.v., $n=4$) in nonanesthetized normotensive rats. Values are expressed as mean $\pm$ SEM. * $P < .05$, ** $P < .01$, *** $P < .001$ versus *Pi*-EtOAc (nonpaired Student's *t*-test).

Pi-EtOAc has no relation to the β -adrenergic receptors. A likely cause for the increase in the hypotensive effect in the intermediate dose can be due to the blockade of the β_1 receptors, which diminishes the response of endogenous catecholamines released in the reflex tachycardia, causing consequently an increase in the hypotensive response of *Pi*-EtOAc. Considering that one of the potential mechanisms causing the hypotension and bradycardia is triggered by the alteration of the efferent parasympathetic tonus of the heart, we checked for the possible indirect effect of *Pi*-EtOAc through the vagus nerve; then, the animals were pretreated with hexamethonium, a ganglionic nicotinic receptors blocker.¹⁰ The *Pi*-EtOAc-induced hypotensive effect was not altered; however, tachycardia in the intermediate doses was attenuated. Somehow, the hypotensive effect was already expected, once the cardiac autonomic blockade did not alter the effect of the drop in ar-

terial pressure and, according to McCallum et al.,²⁴ the sympatholytic central and peripheral effects of the α_2 -adrenergic agonists can be increased with the ganglionic blockade, as was seen in the experiment. In the higher dose, after the blockade, there was attenuation of hypotension followed by tachycardia, suggesting that hypotension and bradycardia in the higher dose without the ganglionic blockade would be a reflex of the chemosensitive hyperstimulation. Thus, the hypotension induced by *Pi*-EtOAc in rats does not seem to be inhibited with the removal of the nervous autonomic support for the cardiovascular system.

Another important factor in the control of the cardiovascular functions is the α_1 -adrenergic receptors that act in the physiological regulations of the vascular smooth muscle tissue when activated by endogenous catecholamines, modulating the vasoconstriction both in the arterial resistance vessel and in the veins, inducing a rise in the blood pressure.²⁵ The α_2 -adrenergic agonists stimulate such receptors of the central and peripheral nervous systems, causing the inhibition of the norepinephrine release by the nervous terminals, diminishing the sympathetic efflux to the periphery. That consequently reduces the vascular peripheral resistance and induces alterations in the cardiac output and bradycardia, resulting in hypotension.²⁶

To verify whether the MAP drop induced by *Pi*-EtOAc would involve the activation of the CNS α_2 -adrenergic receptors, we used yohimbine, a selective antagonist of these receptors.¹² The pretreatment with this pharmacological tool blocked the hypotension induced by *Pi*-EtOAc and induced a probably reflex-related tachycardia. Nevertheless, in the higher dose studied, it completely reverted the hypotensive effect and bradycardia. Hence, *Pi*-EtOAc seems to act similar to the α_2 -adrenergic agonist on the cardiovascular system. These findings are promising due to the lack of reports about the activity of the species *P. insignis* in the cardiovascular system. Nonetheless, additional studies are necessary to provide additional evidences regarding the involved mechanisms as well as the identification of the secondary metabolites responsible for this action.

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AUTHOR DISCLOSURE STATEMENT

The authors declare that they have no competing financial interests.

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