BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902024e23484

Article

Hymenaea rubriflora Ducke stem bark extract has vasorelaxant and contractile inhibition capacity

Keyth Sulamitta de Lima Guimarães[1,5], Luciana Tavares Toscano[1,5], Bagnólia Araújo Costa[2], Iara Leão Luna de Souza[2], Isabelle de Lima Brito Polari[3], Ivyne Oliveira Araújo Wanderley[5], Manoel Miranda Neto[1,5], Bárbara Cavalcanti Barros[2], Rubens Teixeira de Queiroz[4], Ângela Maria Tribuzy de Magalhães Cordeiro[3], Maria da Conceição Rodrigues Gonçalves[1], Lydiane de Lima Tavares Toscano[1,5], Alexandre Sérgio Silva[1,5*]

[1]Post-graduate Program in Nutrition Sciences, Federal University of Paraíba, João Pessoa, Paraíba, Brazil, [2]Post-graduate Program in Natural and Synthetic Bioactive Products, Federal University of Paraíba, João Pessoa, Paraíba, Brazil, [3]Center for Technology and Regional Development, Nucleus LACOM, Federal University of Paraíba, João Pessoa, Paraíba, Brazil, [4]Department of Systematics and Ecology, CCEN, Federal University of Paraíba, João Pessoa, Paraíba, Brazil, [5]Laboratory of Studies on Physical Training Applied to Performance and Health, Department of Physical Education, Federal University of Paraíba, João Pessoa, Paraíba, Brazil

We investigated the vasodilatory effects of *Hymenaea rubriflora Ducke* stem bark extract (HR-HAc). Vascular reactivity of the aortic rings of Wistar rats was tested by *in vitro* cumulative doses (0.1 - 729 µg/mL). Rats (n=5) were treated with 25 (G25), 50 (G50) and 100 (G100) mg/kg of HR-HAc or saline (control group - CG) for four weeks. An *in vitro* assay resulted in dose-dependent relaxation of the aortic rings with functional endothelium, which was inhibited in the presence of L-NAME. Rings of the treated animals increased acetylcholine relaxing potency at all doses, with a greater effect on G50 (pD2 = 7.8±0.1, Emax = 95.6±1.1) and a decreased contractile potency to phenylephrine in G25 (pD2 = 6.9±0.06, Emax = 61.5±6.0%) and G50 (pD2= 6.6±0.06, Emax = 71.0±8.5%) when compared to the CG in the presence and absence of endothelium (pD2= 6.4± 0.1, 6.4±0.1 and 6.9±0.1, respectively). Cumulative doses of nitroprusside resulted in increased relaxing potency in all treated groups and maintained Emax at 100%. It is concluded that HR-HAc has vasorelaxant capacity and inhibitory vascular contraction activity applied either directly to aortic rings or after treatment with *in vivo* supplementation, which places this extract as a potential nutraceutical or pharmacological agent for treating diseases associated with vascular dysfunction.

Keywords: Antioxidant. Aorta. *Hymenaea rubriflora ducke*. Nitric oxide. Vasorelaxation.

INTRODUCTION

Hymenaea is a legume typical of the Brazilian cerrado, also known as "jutaí" or "jatobá of the cerrado" (Silva *et al*., 2019; Veras *et al*., 2020). An ethnopharmacological study indicated that this plant is culturally consumed in South America and Asia, and stem bark infusions are used for medicinal purposes for anemia, retinopathy, sore and respiratory throat (Rocha *et al*., 2018, Silva *et al*., 2019), pain and inflammation (Pacheco *et al*., 2021a).

Dias, Luzia, Jorge (2013) were one of the first to observe that its fruit pulp is rich in minerals, fibers, oils and flavonoids. Its rich nutritional composition and health benefits have already been confirmed in a

***Correspondence:** A. S. Silva. Laboratório de Estudos do Treinamento Físico Aplicado ao Desempenho e a Saúde. Departamento de Educação Física. Centro de Ciências da Saúde, Cidade Universitária. Universidade Federal da Paraíba (UFPB). CEP 58059-900, João Pessoa, Paraíba, Brasil. Phone: +5583988754675. E-mail: alexandresergiosilva@yahoo.com.br. ORCID: https://orcid.org/0000-0003-3576-9023

systematic review (Jacob, Medeiros, Albuquerque, 2020). Furthermore, antioxidant and anti-inflammatory effects of the *Hymenaea courbaril* species have also been shown due to their ability to sequester 2, 2-dipheny l-1-pyrrolhydrazyl (DPPH) radicals, and from the presence of phenolic compounds and linolenic fatty acid (Jacob, Medeiros, Albuquerque, 2020; Pacheco *et al*., 2021b). These characteristics allowed us to raise the hypothesis that *Hymenaea* could be an interesting pharmacological target to develop compounds with vasorelaxant activity. In fact, antioxidant compounds have been demonstrated as presenting vasorelaxant and hypotensive activities (Estrada-Soto *et al*., 2021; Parsamanesh *et al*., 2021; Arias-Durán *et al*., 2021). Similar results have been demonstrated for *in natura* foods rich in phenolic compounds as grapes (Yang *et al*., 2022), strawberries (Ariza *et al*., 2016) and blueberries (Grace *et al*., 2014). *Hymenaea* showed similar total phenolic concentrations to some grape varieties with higher antioxidant capacity (Lutz *et al*., 2011; Yang *et al*., 2022).

Despite this possibility, the vascular relaxing capacity of *Hymenaea* has not yet been tested. In this perspective, the objective of this study was to quantify the total phenolic compound content and the antioxidant capacity of *Hymenaea rubriflora Ducke* (HR-Hac) hydroalcoholic stem bark extract, evaluate the *in vitro* relaxing action of this extract on aortic rings of Wistar rats and to verify the effects of an oral treatment on aortic reactivity to relaxing and contractile agents.

MATERIAL AND METHODS

Preparation of the Hymenaea rubriflora Ducke stem bark extract

The *Hymenaea rubriflora Ducke* specie was collected in the north-eastern region of Brazil and underwent botanical identification of the species by a specialist. An exsicata of the plant was deposited in the Herbarium Profº. Lauro Pires Xavier (JPB)/UFPB under the identification code JPB no. 61262. The stem bark was previously heated in an oven at 50°C. Afterwards, it was subjected to a crushing process using a commercial mixer, followed by porcelain trituration to obtain a final product with 20g of homogeneous particles. The samples were then extracted using a mixture of ethanolic water (1:1) with a frequency of 42kHz for 2h as the solvent, the ratio of powder to solvent volume being 5% (m:v), with a yield of 25% (Bezerra *et al*., 2013).

After the extraction period, the material was vacuum filtered on standard filter paper to remove the particulate material, leaving the filtrate with extractive solution according to the methodology used by Bezerra *et al*. (2013). The extractive solution was heated to 45°C until the volume was reduced to half the initial extraction mixture to obtain the dried extract. Then, the ethanol-free extract was frozen at -80°C for 24h and subsequently lyophilized at 0.024mBar for 24h. The dry extract was obtained as hydroalcoholic extract from the *Hymenaea rubriflora D* (HR-HAc) stem bark. The extract was then stored at -20°C until use in the experiments. A patent application was made for this extracted through the process BR 10 2018 011081 0.

Determination of total phenolic content and antioxidant activity of HR-HAc

The total phenolic content was determined by colorimetric method using the Folin-Ciocalteau reagent (Singleton, Rossi, 1965). The substrate was read at 760nm in a Shimadzu UV-2550 UV-Vis spectrophotometer. The results were expressed as milligram equivalents of gallic acid (GAE) per gram of extract (GAE/g). The antioxidant activity was determined using the methodology described by Brand-Williams, Cuvelier, Berset (1995) through the ability of the antioxidants present in the sample to sequester the stable radical DPPH. The results were expressed in mmol of Trolox per gram of the dried extract (mmol Trolox/g extract).

Animals and ethical aspects

The animals used for the study were male Wistar rats (*Rattus norvegicus*), aged 16 weeks, weighing between 250 and 300g, from Prof. Thomas George from the Institute for Research on Drugs and Medicines

(IPeFarM) at UFPB. The rats were kept in cages with two each, provided a balanced diet ad libitum based on chow (Presence®), free access to water, with controlled and constant ventilation and temperature (21±1°C), submitted daily to a 12-hour light-dark cycle, with the light period being from 6am to 6pm.

All experimental procedures were performed following the principles of animal care Guidelines for ethical animal use (Sherwin *et al*., 2003), and the research project was previously approved by the Ethics Committee in the Use of Animals at IPerFarM under CEUA certificate no. 010/15.

Substances and reagents

The Krebs solution had the following composition: 118.0mM NaCl, 4.6mM KCl, 2.5mM $CaCl_2$, 5.7mM $MgSO_4$, 1.1mM KH_2PO_4, 25.0mM $NaHCO_3$ and 11.0mM glucose. Phenylephrine (Phe), acetylcholine (ACh), sodium nitroprusside (SNP), Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) and indomethacin were purchased from Sigma Aldrich (São Paulo, Brazil). These substances were dissolved and diluted in distilled water to obtain their stock solutions (10^{-2}M), which were maintained at -20°C.

Acute toxicity

A single dose of 2000 mg/kg of HR-HAc was administered per gavage in six rats, followed by monitoring them for 14 days for observation of death, aggressiveness, posture, reaction to manipulation, movements, external appearance, respiration and movement reluctance. The treatment dose and period for assessing acute toxicity followed the methodology described by the Organization for Economic Cooperation and Development (OECD) no. 423/2001 (OECD, 2001), which proposes a guideline for toxicity testing of chemical products.

Oral treatment with HR

A total of 32 rats were supplemented by gavage during four weeks, according to Bezerra *et al*. (2013). The rats were randomly divided into four experimental groups (8 rats/group): G25 (25 mg/kg HR-HAc extract), G50 (50 mg/kg HR-HAc extract), G100 (100 mg/kg extract HR-HAc) and CG (control group, saline). At the end of the treatment period, the rats were euthanized with a guillotine and vascular reactivity tests were performed.

Preparation of aortic rings for reactivity

The thoracic aorta was isolated by laparoscopy, immersed in Krebs solution and gasified with carbogenic mixture (5% CO_2 in O_2) adjusting the pH to 7.4 and temperature of 37°C. The aortic rings (2-3 mm) were exposed to a stabilization period of 60min with a resting tension of 2g. During stabilization, the solution was changed every 15min. After stabilization, a contraction was induced with Phe (3 x 10^{-7} M) and after the contraction tonic phase, ACh (10^{-6} M) was added to verify the integrity of the endothelium (Furchgott, Zawadki, 1980). The vascular endothelium was considered intact when a relaxation equal or greater than 50% was observed (Ajay, Gilani, Mustafa, 2003). Some rings had the endothelium mechanically removed; success was considered by the absence of relaxation induced by ACh or relaxation of less than 10%. The isometric contractions were recorded in isometric force transducers (model TIM-05) coupled to an amplifier model AECAD04F with a BT-60 thermostatic pump system that controlled the temperature of the tanks (AVS Projetos, São Paulo, Brazil).

In vitro assay

After stabilization, aortic rings provided and deprived of endothelium were pre-contracted with Phe ($3x10^{-7}$ M). HR-HAc was cumulatively added (0.1 - 729 μg/mL) in the tonic phase. Other endothelium-provided rings were pre-incubated with L-NAME (10^{-4} M) or indomethacin (10^{-5} M) for 30 minutes prior to pre-contraction with Phe and a subsequent cumulative HR-HAc curve was created. Relaxation was expressed as the reverse percentage of the initial EC50 contraction, expressed as pD2.

Vascular reactivity after oral treatment

Aortic rings were exposed to pre-contraction with Phenylephrine (Phe: $3x10^{7}$M), followed by cumulative relaxation curves to acetylcholine (Ach: 10^{-11}-10^{-4}M) in endothelium-provided rings or sodium nitroprusside (SNP: 10^{-14}-10^{-6}M) for rings without endothelium. The preparations were then washed and after 30 min Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME: 10^{-4}M) was incubated for a period of 30 min and then a new concentration-response curve was induced to the Phe. The reactivity was evaluated by the maximum effect values (Emax) and EC50 (pD2) of the Phe.

Statistical analysis

Data are expressed as mean and standard error of the mean. One-way ANOVA test was used with Bonferroni post-test, when necessary, considering $p<0.05$ as statistically significant. The EC50, Emax and pD2 values were determined by non-linear regression. Statistical analysis was performed using GraphPad Prism® 5.01 program (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

Antioxidant activity, phenolic composition and toxicity of HR-HAc

The antioxidant activity assessment showed high capacity in sequestering the stable free radical (5076.50µmol Tx/g) and phenolic composition (274.63mg ga/g) of HR-HAc extract. The HR-HAc toxicity test showed that there were no risks for the rats, so the three doses were administered safely, since the rats did not show any changes in behavior, clinical signs, food and water consumption patterns, nor variation in body mass.

In vitro assay

When applied directly to the pre-contracted aortic rings with Phe, HR-HAc cumulative doses (0.1 - 729µg/ml) promoted concentration-dependent relaxation in the presence of functional endothelium, as shown in Figure 1, panel A. In this same panel it can be noted that the absence of functional endothelium reduced Emax by 62%, and increased EC50. The relaxation was also attenuated in the presence of L-NAME, in rings with functional endothelium, as shown in Figure 1, panel B. Meanwhile, the presence of indomethacin maintained Emax, but significantly decreased EC50 in rings with functional endothelium (Figure 1, panel C). Aortic responsiveness was reestablished 30 minutes after the HR-HAc (data not shown).

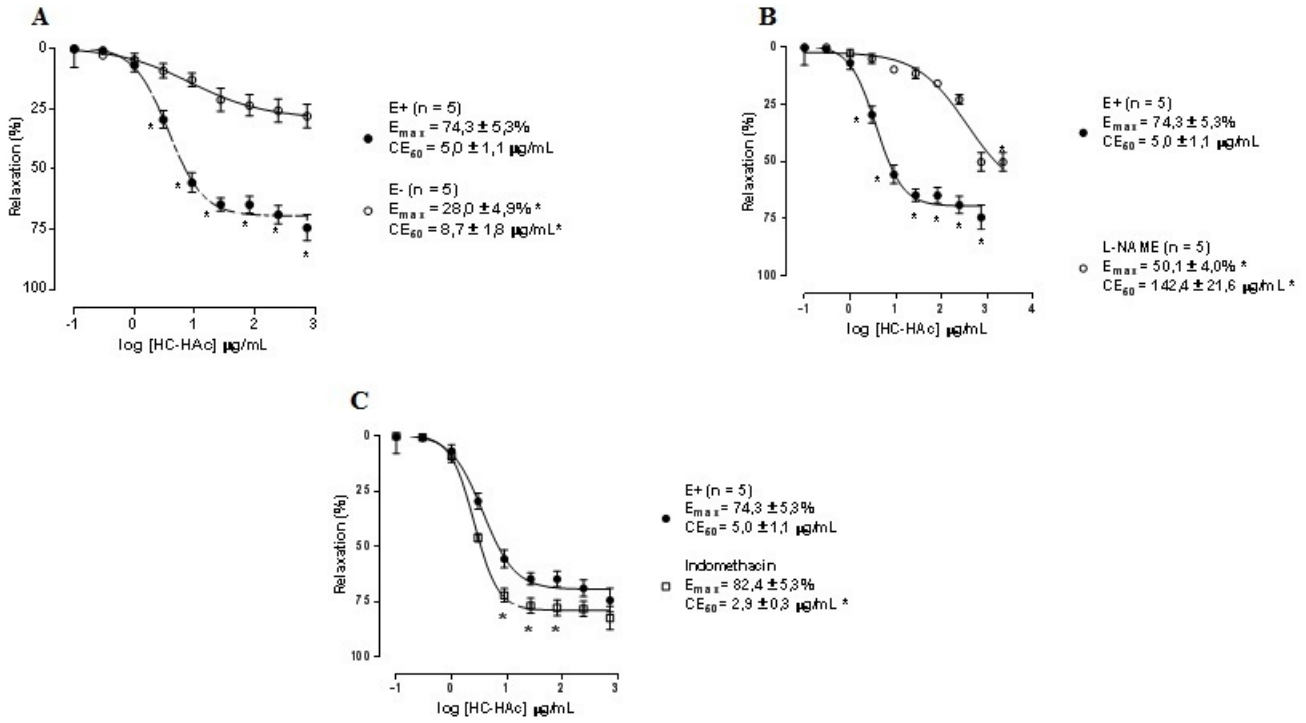

FIGURE 1 - Vasorelaxant effect of the HR-HAc on aortic rings pre-contracted with phenylephrine (Phe, 3 x 10-7 M), in the presence (E+) and absence (E-) of endothelium-intact (panel A), L-NAME (10-4 M, panel B) and indomethacin (10-5 M, panel C). Data are mean ± S.E.M. (n=5). * $p<0.05$ by One-way ANOVA followed by Bonferroni multiple comparisons test vs presence of endothelium-intact (E+).

Effect of HC-HAc on vascular reactivity to AcH after treatment with HC

As shown in Figure 2, panel A, increased relaxing potency for ACh in functional endothelium aorta rings of the rats treated with 50 mg/kg and 100 mg/kg compared to saline (control) is observed. The relaxing potency in rings of rats treated with 25 mg/kg HC-HAc was similar to the control group. Increased relaxant potency was observed despite the supplementation not affecting Emax in any of the treated groups compared to the control.

Panel B of Figure 2 shows that cumulative doses of SNP resulted in an increase in the relaxing potency in all groups when compared to ACh curve. Emax was maintained at 100%, with the groups treated with 25 mg/kg, 50 mg/kg and 100 mg/kg. HC-HAc exhibited significantly higher pD2 than the control group and rats treated with 100 mg/kg showed significant greater relaxing response than other groups.

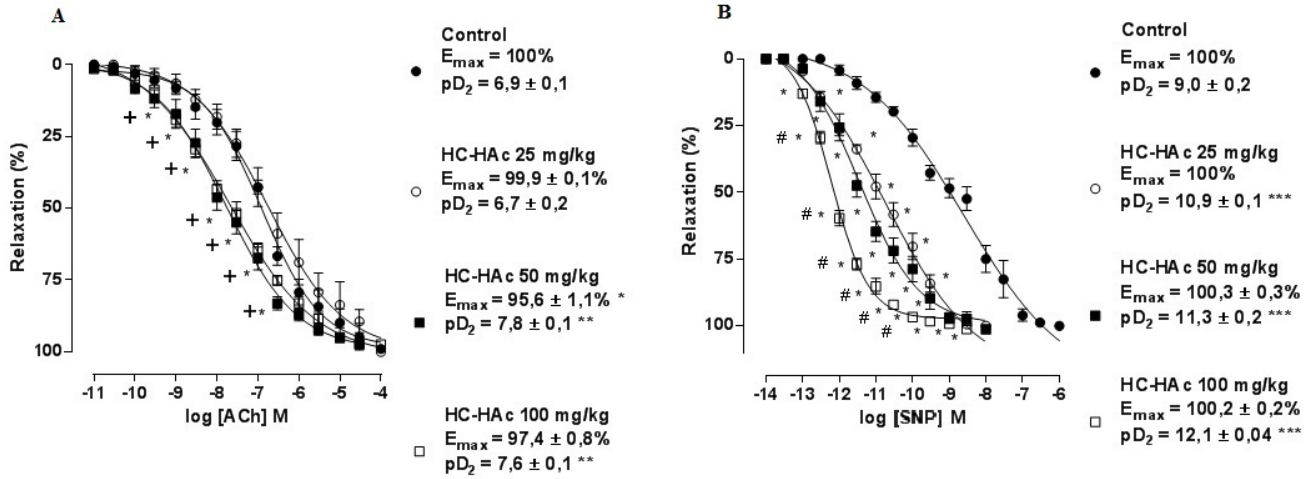

FIGURE 2 - Relaxation of aortic rings induced by ACh (panel A) and SNP (panel B) in rats. Control group (˜), rats treated with HR-HAc 25 mg/kg (™), 50 mg/kg (¢) and 100 mg/kg (£). Data are mean ± S.E.M. (n=5)* $p<0.05$ vs control (Two-way ANOVA); † $p<0.05$ vs HR-HAc 25 mg/kg (One-way ANOVA followed by Bonferroni multiple comparisons test).

CONTRACTILE RESPONSE TO PHE IN HC-TREATED ANIMALS

It is shown in Figure 3 (panel A) that supplementation with HR-HAc at the dose of 25mg/kg did not alter the contractile potency of the Phe in the aorta in relation to control in the presence of functional endothelium, however this dose of 25 mg/kg caused an Emax reduction. Meanwhile, treatment at doses of 50 mg/kg and 100 mg/kg, deviated the concentration-response curve to the right and reduced the contractile power of the Phe.

The contractile potency for Phe in rings without functional endothelium in rats supplemented with HR-HAc at doses of 25 mg/kg, 50 mg/kg and 100 mg/kg was reduced in relation to the control group, but without differences between the three groups treated with HR-HAc (Figure 3, panel B). It was further observed that G25 maintained maximum response regarding Emax without differences for the CG, however this was reduced at doses of 50 mg/kg and 100 mg/kg.

In the experiments performed after pre-incubattion with L-NAME, rings of the rats that received 25 mg/kg, 50mg/kg and 100 mg/kg showed a significant reduction in the contractile power and the maximum response in relation to the control group (Figure 3, panel C). The effects of the natural agent obtained from *Hymenaea rubriflora Ducke* bark on the vascular function of Wistar rats is shown in Figure 4.

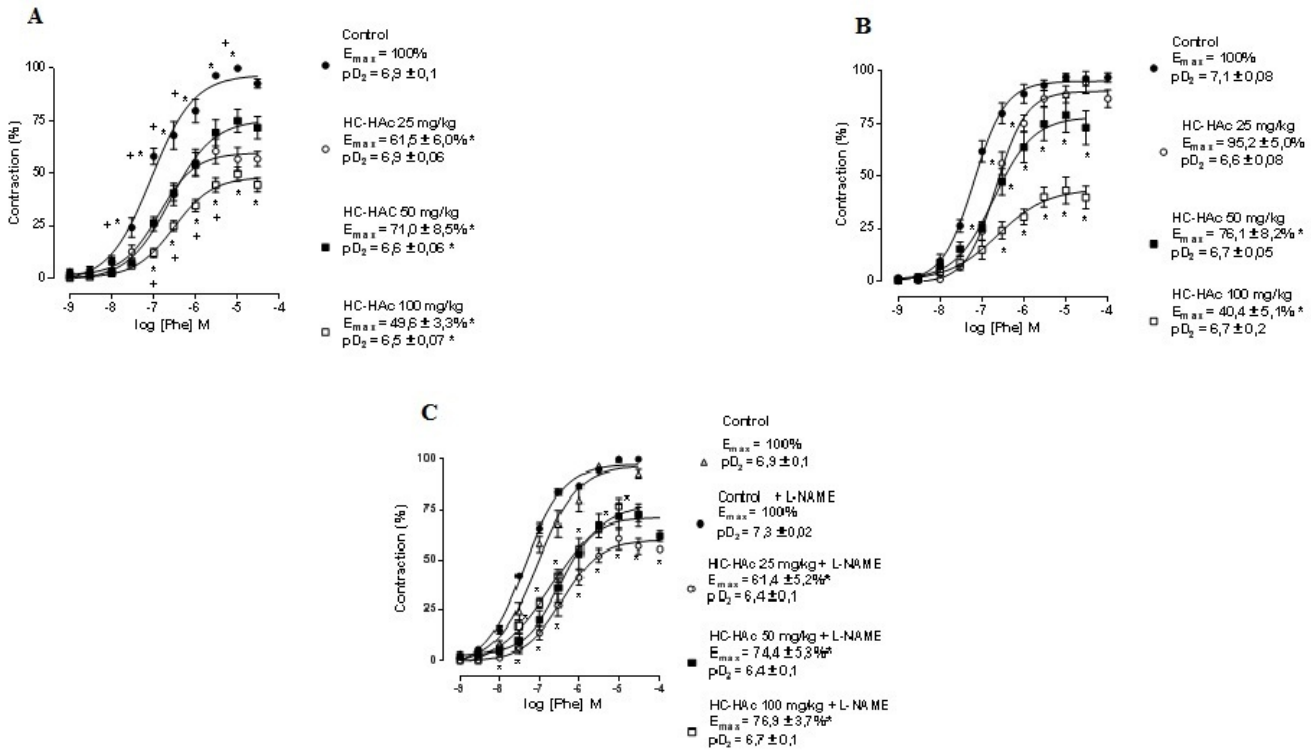

FIGURE 3 - Contractile effect of the Phe in the presence (panel A) or absence of endothelium-intact (panel B), L-NAME (10-4M, panel C) in rats treated with HR-HAc (25, 50 and 100mg/kg). Data are mean ± S.E.M. (n=5). * $p<0.05$ vs control (Two-way ANOVA); † $p<0.05$ vs HR-HAc 25 mg/kg (One-way ANOVA followed by Bonferroni multiple comparisons test).

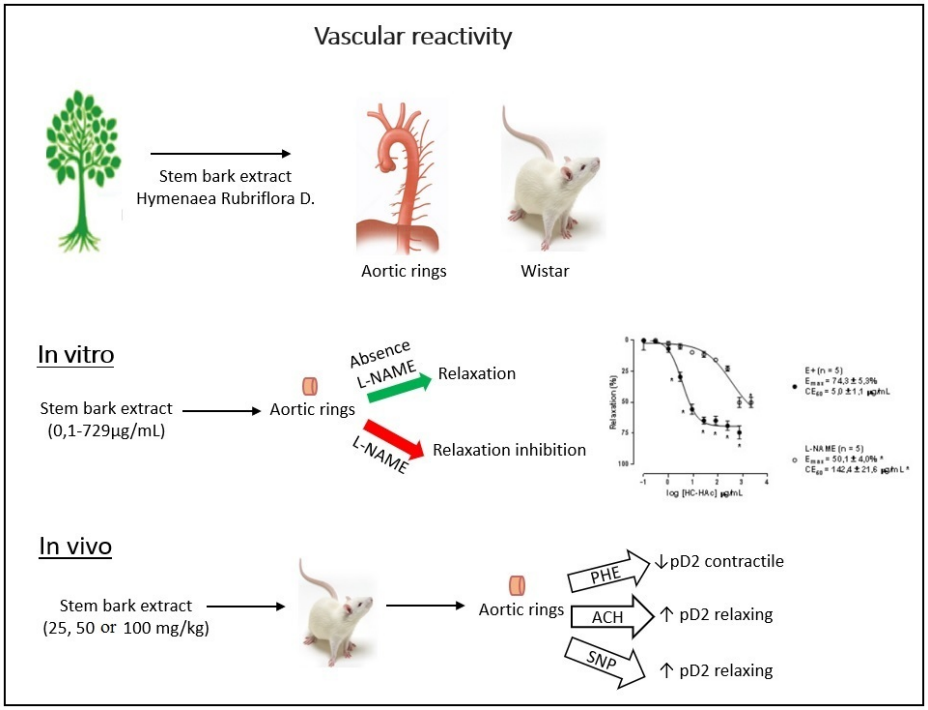

FIGURE 4 - Graphical abstract. Effect of natural agent obtained from *Hymenaea rubriflora Ducke* bark on the vascular function of Wistar rats.

DISCUSSION

The data from this study lead to three findings: (1) *Hymenaea rubriflora Ducke* stem bark is rich in antioxidants; (2) Antioxidant compounds have relaxing activity *in vitro*; (3) The relaxing activity *in vitro* has potential for future clinical application because it was maintained after treatment with oral ingestion in rats.

Regarding the first finding, the antioxidant properties of the stem bark had previously been demonstrated in other *Hymenaea* species, such as *courbaril* (Jacob, Medeiros, Albuquerque, 2020), *stigonocarpa Mart* (Pimentel *et al*., 2023) and *martiana Hayne* (Pacheco *et.al*., 2021b), mainly attributed to the presence of procyanidins and carotenoids such as beta-carotene and lutein. *Hymenaea rubriflora Ducke* characterization in the present study confirms the composition of antioxidant compounds in *Hymenaea*. However, it should be noted that we found greater antioxidant activity in the stem bark rather than the fruit or fruit skin. In fact, infusions obtained from the stem bark have been used for medicinal purposes in popular culture (Cartaxo, De Almeida, De Albuquerque, 2010; Rocha *et al*., 2018; Veras *et al*., 2020).

Regarding the second finding, *in vitro* antioxidant action has already been demonstrated in previous studies (Bezerra *et al*., 2013; Veras *et al*., 2020; Scaramussa, Soares, Santana, 2022). The unprecedented finding of this study, at least as far as we know, was that HR-HAc antioxidant compounds have the ability to promote vasodilator effects and inhibit contractile and artery activity in rats. Previous studies have shown that *Hymenaea* species had the ability to promote myorelaxant (Bezerra *et al*., 2013) and anti-inflammatory (Pacheco *et al*., 2021a) activities, including inhibitory activity of 5-lipoxygenase *in vitro* (Braga *et al*., 2000) and anti-inflammatory gastroprotective effect (Orsi *et al*., 2012; Martins *et al*., 2015). In fact, both oxidative stress and inflammation primarily participate in the etiology of vascular diseases that involve vasoconstriction and inhibition of relaxing activity (Tan *et al*., 2013).

The vasodilator capacity discovered in the *in vivo* experimental level provides a promising avenue for exploring this vegetable for treating arterial hypertension, the most associated disease related to vascular dysfunction. The HR-HAc did not present acute toxicity when administered in a single dose of 2000 mg/kg following Acute Oral toxicity (OECD, no.423 - 2001), corroborating with a toxicity test of the *courbaril* species (Bezerra *et al*., 2013). Thus, doses of 25 (G25), 50 (G50) and 100 (G100) mg/kg provided supplementation safety for the animals, since up to the present study there were no supplementation protocols for this extract. This data ratifies our understanding of the potential of HR-HAc for the evolution of the research to the clinical level.

Absence of the relaxant effect when the experiments were performed on rings devoid of endothelium indicate that the vasorelaxant action is endothelium-dependent. Experimental studies have shown the participation of phenolic compounds in vascular modulation via the endothelial route (Toscano *et al*., 2014; Jacob, Medeiros, Albuquerque, 2020; Parsamanesh *et al*., 2021) and in the promotion of vasodilation and blood pressure reduction (Cartagenes *et al*., 2014). Meanwhile clinical studies indicated a reduction in blood pressure, acting on the improvement of endothelial function (Toscano *et al*., 2014; Le Sayec *et al*., 2022). In addition, it has been demonstrated by trials with prostacyclin inhibitors that the relaxing effect appears to be nitric oxide dependent. The clinical significance of this finding is that endothelial dysfunction, particularly because of inability to nitric oxide production, is one of the main characteristics of cardiovascular diseases in humans, such as arterial hypertension (Saenz-Medina *et al.,* 2022).

There was still some vascular relaxation in the rings pre-incubated with L-NAME, an inhibitor of nitric oxide synthesis, indicating that some other mechanism has small participation in the vasorelaxant action. A study by Bezerra *et al*. (2013) demonstrated the ability of the *H. courbaril* hydroalcoholic extract at a dose of 150 mg/kg to inhibit approximately 50% of the K^+ induced contraction in tracheal rings of ovalbumin-hypersensitive rats. However, we did not investigate this mechanism in the aortic rings in the present study, so we are warned of this need in future studies.

The contractile efficacy of Phe at a dose of 100 mg/kg was reduced in absence of the functional endothelium, which demonstrates that the inhibition of the contractile activity promoted by the HR-HAc extract is also

dependent on the vascular endothelium. However, in the presence of L-NAME, the contractile potency of Phe was similar to that obtained in the absence of this inhibitor in the G50 and G100 groups, suggesting that nitric oxide probably is not the main substance responsible for the decrease in the contractile activity of the aorta.

Together, these findings point to HR-HAc as a postulant therapeutic tool for diseases associated with vascular dysfunction because of its vasorelaxant capacity and contractile inhibition properties, without adverse effects.

An important limitation of this study is that we hypothesized that the vasorelaxative action of HR-HAc would be due to the rich phenolic content, however we did not quantify the antioxidant effect in the aorta of the animals. Therefore, for future studies, we recommend checking the association of vasodilation with possible better antioxidant activity in the vessels. We also recommend carrying out other tests aimed at verifying endothelium-independent mechanisms in detail which were shown to be possibly involved in the vasomotor response in this study, but which were not investigated. We also recommend investigating participation of hyperpolarizing factors derived from the endothelium. Molecular assays are needed to verify the participation of these compounds in stimulating nitric oxide production, as well as histological assays to verify if the compounds of *Hymenaea* influence the vascular structures. We recommended to repeat the tests on resistance vessels, such as femoral and mesenteric, since the properties of these vessels are different from those of aortic vessels. Finally, studies should be carried out to evaluate the effect of HR-HAc on blood pressure in hypertensive rat models in order to better evaluate the possibility that we will have a new potential nutraceutical or pharmacological agent for treating hypertension in clinical studies.

CONCLUSION

The data of this study demonstrated that *Hymenaea rubriflora Ducke* stem bark has high antioxidant composition and an extract elaborated from this stem has both endothelium-dependent vasorelaxant and inhibitory capacity of the vascular contraction in the aorta of Wistar rats. These data raise the prospect of being able to develop a new antihypertensive agent with this extract.

ACKNOWLEDGMENTS

This study was supported by the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (Brazilian Federal Agency for the Support and Evaluation of Graduate Education; CAPES, Brazil).

CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication.

FUNDING

This study did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

ETHICS STATEMENTS

The Ethics Committee on the Use of Animals of Universidade Federal da Paraíba approved the animal protocol (Approval number: 010/2015). The animals were maintained in accordance with the guidelines of Guidelines for the ethical use animals in applied etiology studies (Sherwin *et al.*, 2003).

REFERENCES

Ajay M, Gilani A, Mustafa M. Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. Life Sci. 2003;129:603-612.

Arias-Durán L, Estrada-Soto S, Hernández-Morales M, Millán-Pacheco C, Navarrete-Vázquez G, Villalobos-Molina R, et al. Antihypertensive and vasorelaxant effect of leucodin and achillin isolated from Achillea millefolium through calcium channel blockade and NO production: In vivo, functional ex vivo and in silico studies. J Ethnopharmacol. 2021;12(273):1-10.

Ariza MT, Reboredo-Rodríguez P, Mazzoni L, Forbes-Hernández TY, Giampieri F, Afrin S, et al. Strawberry

Achenes Are an Important Source of Bioactive Compounds for Human Health**.** Int. J. Mol. Sci. 2016;17:1103.

Bezerra GP, Da Silva Góis RW, De Brito TS, De Lima FJB, Bandeira MAM, Romero NR, et al. Phytochemical study guided by the myorelaxant activity of the crude extract, fractions and constituent from stem bark of Hymenaea courbaril L. J. of ethnopharm 2013;149(1):62-69.

Braga FC, Wagner H, Lombardi JA, De Oliveira AB. Screening Brazilian plant species for in vitro inhibition of 5-lipoxygenase. Phytomedicine. 2000;6(6):447-452.

Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT – Food sci and Techn. 1995;28(1):25-30.

Cartagenes MSS, Lima NFMC França LG, Pessoa DLR, Amaral FMM, Abreu IC, et al. Avaliação Da Atividade Anti-Hipertensiva Do Extrato De Arrabidaeachica Verlot Em Ratos Espontaneamente Hipertensos. Rev. Ciênc. Saúde. 2014;16(2):98-105.

Cartaxo SL, De Almeida MMS, De Albuquerque UP. Medicinal plants with bioprospecting potential used in semi-arid northeastern Brazil. J. of ethnopharm. 2010;131(2):326-342.

Dias LS, Luzia DMM, Jorge N. Physicochemical and bioactive properties of Hymenaea courbaril L. pulp and seed lipid fraction. Industrial Crops and Products. 2013;49:610-618.

Estrada-Soto S, González-Trujano ME, Rendón-Vallejo P, Arias-Durán L, Ávila-Villarreal G, Villalobos-Molina R. Antihypertensive and vasorelaxant mode of action of the ethanol-soluble extract from Tagetes lucida Cav. aerial parts and its main bioactive metabolites. J Ethnopharmacol. 2021;10(266):1-8.

Furchgott R, Zawadzki J. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980;288:373-376.

Grace MH, Esposito D, Dunlap KL, Lila MA. Comparative analysis of phenolic content and profile, antioxidant capacity, and anti-inflammatory bioactivity in wild Alaskan and commercial Vaccinium berries. J Agric Food Chem. 2014;62(18):4007-4017.

Jacob MCM, Medeiros MFA, Albuquerque UP. Biodiverse food plants in the semiarid region of Brazil have unknown potential: A systematic review. PLoS One. 2020;7:15(5):1-24.

Le Sayec M, Xu Y, Laiola M, Gallego FA, Katsikioti D, Durbidge C , et al. The effects of Aronia berry (poly)phenol supplementation on arterial function and the gut microbiome in middle aged men and women: Results from a randomized controlled trial. Clin Nutr. 2022;41(11):2549-2561.

Lutz M, Jorquera K, Cancino B, Ruby R, Henriquez C. Phenolics and antioxidant capacity of table grape (Vitis vinifera L.) cultivars grown in Chile**.** J Food Sci. 2011;76:C1088-C1093.

Martins JLR, Rodrigues ORL, Sousa FB, Fajemiroye JO, Galdino PM, Florentino IF, et al. Medicinal species with gastroprotective activity found in the Brazilian Cerrado. Fundam Clin Pharmacol. 2015;29(3):238-51.

OECD (Organization for Economic Cooperation and Development). Acute oral toxicity – Acute toxic class method. OECD guideline for testing of chemicals n° 423, 2001.

Orsi PR, Bonamin F, Severi JA, Santos RC, Vilegas W, Hiruma-Lima CA, et al. Hymenaea stigonocarpa Mart. ex Hayne: A Brazilian medicinal plant with gastric and duodenal anti-ulcer and antidiarrheal effects in experimental rodent models. J of Ethnophar. 2012;143(1):81-90.

Pacheco AGM, Pacheco EJ, Macedo LARO, Silva JC, Lima-Saraiva SRG, Barros VP,

et al. Antinociceptive and anti-inflammatory activities of Hymenaea martiana Hayne (Fabaceae) in mice. Braz J Biol. 2021a;2(82):1-9.

Pacheco AGM, Branco A, Camara CA, Silva TMS, Silva TMG, De Oliveira AP, et al. Identification of flavonoids in Hymenaea martiana Hayne (Fabaceae) by HPLC-DAD-MSn analysis. Natural product research. 2021b;35(14):2414-2419.

Parsamanesh N, Asghari A, Sardari S, Tasbandi A, Jamialahmadi T, Xu S, et al. Resveratrol and endothelial function: A literature review. Pharmacol Res. 2021;170:1-14.

Pimentel FC, Alves CCF, Forim MR, Matos AP, Cunha GOS, Cazal CM. Chemical composition and antifungal activity of the essential oil from the *Hymenaea stigonocarpa* Mart. Ex Hayne (jatobá-do-cerrado) fruit peel. Nat Prod Res. 2023;16:1-5.

Rocha LS, Gonçalves DA, Arakaki DG, Tschinkel PFS, De Lima NV, De Oliveira LCS, et al. Data on elemental composition of the medicinal plant Hymenaea martiana Hayne (Jatobá). Data in brief. 2018;19: 959-964.

Saenz-Medina J, Muñoz M, Rodriguez C, Sanchez A, Contreras C, Carballido-Rodríguez J, et al. Endothelial Dysfunction: An Intermediate Clinical Feature between Urolithiasis and Cardiovascular Diseases. Int J Mol Sci. 2022;14;23(2):912.

Scaramussa SAL, Soares LA, Santana LCLA. Extracts from jatobá (*Hymenaea courbaril* L.) peel and seeds: Antioxidant and antimicrobial activities and synergistic effect of extract combinations. Food Sci Technol Int. 2022;4:1-9.

Silva CP, Soares-Freitas RAM, Sampaio GR, Santos MCB, Nascimento TP, Cameron LC, et al. Identification

and action of phenolic compounds of Jatobá-do-cerrado (Hymenaea stignocarpa Mart.) on α-amylase and α-glucosidase activities and flour effect on glycemic response and nutritional quality of breads. Food Res Int. 2019;116:1076-1083.

Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965;16:144-158.

Sherwin CM, Christiansen SB, Duncan IJ, Erhard HW, Lay JRDC, Mench JA, et al. Guidelines for the ethical use of animals in applied ethology studies. Applied Anim Beha Sci. 2003;81(3):291-305.

Tan VP, Chung A, Yan BP, Gibson PR. Venous and arterial disease in inflammatory bowel disease. J Gastroenterol Hepatol. 2013;28(7):1095-113.

Toscano LT, Silva CSO, Toscano LT, Almeida AEM, Santos AC, Silva AS. Chia flour supplementation reduces blood pressure in hypertensive subjects. Plant Foods Hum Nutr. 2014;69(4):392-8.

Veras BO, Oliveira MBM, Oliveira FGS, Santos YQ, Oliveira JRS, Lima VLM, et al. Chemical composition and evaluation of the antinociceptive, antioxidant and antimicrobial effects of essential oil from Hymenaea cangaceira (Pinto, Mansano & Azevedo) native to Brazil: A natural medicine. J Ethnopharmacol. 2020;30(247):112265.

Yang C, Han Y, Tian X, Sajid M, Mehmood S, Wang H, et al. Phenolic composition of grape pomace and its metabolism. Crit Rev Food Sci Nutr. 2022;17:1-17.

Received for publication on 01st August 2023
Accepted for publication on 10th October 2023