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Original Article

In vivo bronchodilator evaluation of the ethanolic extract of the stems of *Jatropha macrantha* Müll.Arg.

[Evaluación broncodilatadora *in vivo* del extracto etanólico de los tallos de *Jatropha macrantha* Müll.Arg.]

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Abstract

Context: Asthma is characterized by hyperresponsiveness and bronchoconstriction, induced by histamine and other noxes, with a prevalence in Peru of 20.7-28.2%, which is why, in traditional medicine, *Jatropha macrantha* Müll.Arg is used.

Aims: To evaluate the *in vivo* bronchodilator activity of the ethanolic extract of the stems of *J. macrantha*.

Methods: The powdered material of the stem of *J. macrantha* was extracted with ethanol 96° by maceration; then, the phytochemical screening was carried out. The doses of 200, 400 and 800 mg/kg of the extracts were subjected to *in vivo* evaluation in guinea pigs (*Cavia porcellus*) with 1% histamine-induced bronchoconstriction, determining the latency period and the intensity of the effect.

Results: Phytochemical sieving showed alkaloids, catechins, flavonoids, tannins and terpenes. The activity was observed at the three doses of the *J. macrantha* extract, being higher at a single dose of 400 mg/kg, with a latency period of 33.0 ± 1.02 s, asphyxia 84.5 ± 1.08 s and anaphylaxis 105.6 ± 0.59 s. The analysis of variance and Dunnett's test resulted with $p<0.05$ as statistically significant.

Conclusions: The results showed bronchodilator activity of the ethanolic extract of *J. macrantha* at an average dose of 400 mg/kg, orally, in *Cavia porcellus*.

Keywords: *Jatropha macrantha*; huanarpo female; bronchodilator activity.

Resumen

Contexto: El asma se caracteriza por la hiperreactividad y la broncoconstricción, inducida por la histamina y otras noxas, con una prevalencia en el Perú del 20,7-28,2%, por lo que, en la medicina tradicional, se utiliza la *J. macrantha*.

Objetivos: Evaluar la actividad broncodilatadora *in vivo* del extracto etanólico de los tallos de *J. macrantha*.

Métodos: El material en polvo del tallo de *J. macrantha* se extrajo con etanol 96° por maceración; luego, se realizó el tamizaje fitoquímico. Las dosis de 200, 400 y 800 mg/kg de los extractos se sometieron a evaluación *in vivo* en cobayos (*Cavia porcellus*) con broncoconstricción inducida con histamina 1%, determinándose el período de latencia y la intensidad del efecto.

Resultados: Al tamizado fitoquímico se observó alcaloides, catequinas, flavonoides, taninos y terpenos. Se observó actividad a las tres dosis del extracto de Jatropha, siendo mayor a dosis única de 400 mg/kg, con un período de latencia de 33,0 ± 1,02 s), asfixia 84.5 ± 1,08 s y anafilaxia 105,6 ± 0,59 s. El análisis de varianza y la prueba de Dunnett's resultó con $p<0,05$ como estadísticamente significativo.

Conclusiones: Los resultados mostraron actividad broncodilatadora del extracto etanólico de tallos de *J. macrantha* a una dosis media de 400 mg/kg, por vía oral, en *Cavia porcellus*.

Palabras Clave: *Jatropha macrantha*; huanarpo hembra; actividad broncodilatadora.

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INTRODUCTION

Acute respiratory infections (ARI) are diseases of the respiratory system caused by bacteria, viruses, and other etiological agents (Córdova et al., 2020); being asthma one of the chronic diseases of the lower respiratory tract, with a prevalence of 6 to 30% throughout the world (Mallol et al., 2013; Cotrina et al., 2020; Del Rio-Navarro et al., 2020), and in Peru, it is from 20.7 to 28.2%, the same that decreases in higher altitude areas (Vargas et al., 2018; Cotrina et al., 2020). Asthma is characterized by hyperresponsiveness and bronchoconstriction that limit airflow, its molecular mechanism involves allergens and other toxins that interact with the respiratory epithelium, producing pro-inflammatory cytokines such as periostin, thymic stromal lymphopoietin (TSLP) and interleukins (IL-5, IL-13, IL-25, IL-33), which activate dendritic cells (DC), which promote the differentiation of naive $CD4^+$ T cells into T_H2 lymphocytic cells (Paul and Zhu, 2010; Durán, 2015) with the participation of transcription factors, STAT6 and GATA3 (Wan, 2014); T_H2 release cytokines from type 2 [interleukin (IL-4 and IL-13)] that differentiate and activate B lymphocytes (Durán, 2015; Fahy, 2015), which produce IgE, which, when interacting with IgE receptors on mast cell membranes, generate the release of histamine (stimulate H_4 receptors), leukotrienes LTC_4 and LTD_4 (stimulate cysteinyl-LT1 receptors), prostaglandin D_2 (PGD_2), and chymases, responsible for the contraction and hyperresponsiveness of bronchial smooth muscle (Durán, 2015; Wendell et al., 2020). Innate group 2 lymphoid cells (ILC2) (Wendell et al., 2020) produce type 2 cytokines (IL-4, IL-5, and IL-13) (Chen et al., 2017) and contribute to the differentiation of naive $CD4^+$ T cells into TH_2 cells (Wendell et al., 2020). It is important to understand the molecular mechanisms of asthma, to study metabolites of medicinal plants with possible mechanisms of action of antagonizing receptors or inhibiting the release of the pro-inflammatory drugs described. It is known that, since the dawn of medicine, plants have been used as food and for the treatment of diseases, and today, technology is isolating their metabolites and elucidating their chemical structure, to promote them for therapeutic purposes, according to the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), it is estimated that two-thirds of the world's population use plants as a therapeutic alternative (Gallegos, 2016). Due to its climatic diversity, the Peruvian territory is possessed of abundant plant resources, growing the genus *Jatropha* (Greek word *jatros*, medical; *trophe*, food), which belongs to the *Euphorbiaceae* family, with approximately 199 known species (Sabander et al., 2013).

Jatropha species have been identified as containing monoterpenes, diterpenes, triterpenes, sesquiterpenes, cyclic peptides, lignans, neoligns, sesquineolignas, flavonoids, coumarins, coumarin lignans, alkaloids and eudesmenoic acids (Cavalcante et al., 2020). One of these species is the *Jatropha macrantha* Müll.Arg. (female huanarpo) that contains in its chemical composition, pomolic acid and euscaphic acid (Apaza et al., 2020); alkaloids, essential oils, steroids, flavonoids, saponins, and catechins (Tinco et al., 2011). In another study, the stems of *J. macrantha* stems were reported to contain two catechins, 7-O-β-glucopyranosyl-catechin and proanthocyanidin β-3 (Benavides et al., 2006). Traditional preparations based on stems of *J. macrantha* are used for the treatment of bronchitis, asthma and other conditions (Apaza et al., 2020).

After conducting a review in the PubMed-NCBI database on the scientific literature on the effects of *J. macrantha* in Peru, it has been shown that studies on this plant are still scarce, so it has been decided to evaluate the potential bronchodilator effect of the ethanolic extract of the stem of *J. macrantha*, which is part of the traditional medicine of the inhabitants of the Socco annex, Tibillo district of the Palpa province, Ica region, to validate popular knowledge and contribute scientific evidence on its bioactive properties.

The present study aimed to evaluate the bronchodilator activity of the ethanolic extract of the stems of *J. macrantha*, *in vivo* (*Cavia porcellus*), by

the method of histamine-induced bronchoconstriction.

MATERIAL AND METHODS

Type of study

A double-blind, analytical and experimental study was carried out.

Study period

The study was carried out from November 2019 to April 31, 2021.

Study population or experimental animals

Intentional and convenience sampling of 20 male guinea pigs (*Cavia porcellus*) weighing between 700 ± 100 g.

The guinea pigs were acquired from the Bioterium of the National Center for Biological Products (CNPB) of the National Institute of Health. They were then transferred to the pharmacology laboratory of the Faculty of Pharmacy and Biochemistry of the San Luis Gonzaga National University. The 20 guinea pigs were housed in clean and well ventilated propylene cages and kept under standardized laboratory conditions (12 h light/dark cycle, 24°C). They were provided adequate food for their species and drinking water *ad libitum*. The animals were acclimatized for two weeks, monitoring their weight every two days. After that time the experiment began.

Vegetable sample

The *Jatropha macrantha* Müll.Arg. "female huanarpo" is a small shrub, whose flowering and fruitful branches were collected in November 2019 in the Socco annex, Tibillo district (**g**eographically it is located between 14°24′59″S 75°12′23″W; 2167 masl), Palpa province, Ica region, southern Peru. The plant was identified by Dr. Berta Loja Herrera (Taxonomic Biologist), reviewing the specialized bibliography, analyzing the vegetative and reproductive characters, based on the descriptions and taxonomic keys valid for contrasting and determining the taxa (Cerrate, 1969; Soukup, 1987; Valdizán and Maldonado, 1992). A specimen of the plant was deposited in the Herbarium of the Natural History Museum of the National University of San Marcos, Lima, Peru, certificate N° 81-USM-2014.

Preparation of the plant extract

The dried stems were pulverized in an artisanal disk mill until obtaining a semi-fine powder, then 10 g were weighed and macerated for 14 days at room temperature, with two liters of 96° ethanol, under stirring for 4 hours per day. Filtration was carried out with a high precision vacuum pump (Fisatom model 920, Brazil), using Whatman paper Nº 1 (Merck), separating 2 mL whose solution was called fraction A, the rest of the extract was concentrated to dryness and reduced pressure using a rotary evaporator (Heidolph model LABOROTA 4000, Germany) at a temperature of 40°C, a fraction was separated for phytochemical screening (A1), and the largest quantity was brought to the oven (Binder series 05-75803, Germany) at 40°C to evaporate the solvent. The dry extracts were preserved in amber flasks at refrigeration temperature (4°C) until the animal experiments were carried out.

Phytochemical screening

Phytochemical screening was carried out according to the methodology reported by Lock (2016), identifying secondary metabolites through coloration and/or precipitation reactions in solvents of different polarities. In the fraction A, free amino acids, flavonoids, phenols and tannins were identified, while a 1% HCl solution was added to fraction A1, stirring, then filtered and an insoluble part and an acid solution were obtained. The insoluble part was washed with distilled water to neutral pH. Subsequently, it was dissolved with 5 mL of dichloromethane, dried with anhydrous sodium sulfate and filtered, obtaining fraction B (with it, terpenes were identified), the acid solution was filtered, adding 25% ammonium hydroxide, and the extraction was carried out with dichloromethane, forming two phases, the dichloromethane and the aqueous one. The dichloromethane phase was washed with distilled water, dried with anhydrous sodium sulfate and

immediately filtered to obtain fraction C (alkaloids, coumarins, lactones and terpenes were identified); while the aqueous phase was saturated with 5 g of anhydrous sodium sulfate, carrying out the extraction with dichloromethane:ethanol (3:2), generating an organic and an aqueous phase. The organic phase was washed with anhydrous sodium sulfate solution (the aqueous residue was saved) and the organic part was dehydrated with 1 g of anhydrous sodium sulfate, filtering to obtain fraction D (alkaloids, catechins, coumarins, flavonoids were identified and terpenes); while the aqueous residue was added to the aqueous phase (the result of the dichloromethane: ethanol extraction), generating fraction E (catechins and flavonoids were identified). Additionally, 1 g of the dry drug was mixed with 20 mL of distilled water, stirred and boiled for 15 minutes, then filtered hot and made up to volume (10 mL) through the filter, allowed to cool to room temperature, constituting fraction F and with it, saponins were identified (Lock, 2016)**.** **T**he reagents used are mentioned in **T**able 2.

Design of the experiment

After the two weeks of acclimatization, the guinea pigs were weighed and five groups were each formed with four experimental animals, to which a single dose was administered at a concentration of 200, 400 and 800 mg/kg of weight corporal, the same that were sustained in the previous studies of Aguilar (2015). The study design is presented in Table 1.

Assessment of bronchodilator activity

The method of histamine-induced bronchoconstriction was used in guinea pigs *in vivo*, which is a traditional immunological model of antigen-induced airway obstruction. The parameters to be evaluated were the latency period (respiratory distress observed with the first cough) and the intensity of the effect (asphyxia due to tracheal occlusion and anaphylaxis). For this, 30 min before the experiment, the extracts and controls were administered in a single dose, after that time, all groups were subjected to nebulization of 1% histamine in an airtight chamber for 30 s at a pressure of 8 L/min (Zárate et al., 1999; Aguilar, 2015).

The animals that, after the experiment, remained in poor condition with signs of severe or chronic pain, or distress, were sacrificed with an overdose of sodium pentobarbital intraperitoneally (100 mg/kg). The dead animals were discarded according to NTS No 144-MINSA/2018/DIGESA, Technical Health Standard "Comprehensive Management and Solid Waste Management in Health Establishments, Support Medical Services and Research Centers."

Table 1. Design of administration of controls and *J. macrantha* extract in experimental animals.

Group	Plant drug/drug/placebo	Animal treatment
G-1	Physiological saline	The negative control group was administered physiological saline (2 mL/kg of body weight) by means of a gastric gold tube.
G-2	*J. macrantha* 1 extract	*J. macrantha* extract was administered at a dose of 200 mg/kg of body weight by means of a gastric gold tube.
G-3	*J. macrantha* 2 extract	*J. macrantha* extract was administered at a dose of 400 mg/kg of body weight by means of a gastric gold tube.
G-4	*J. macrantha* 3 extract	*J. macrantha* extract was administered at a dose of 800 mg/kg of body weight by means of a gastric gold tube.
G-5	Chlorphenamine	Chlorphenamine was administered to the positive control group at a dose of 10 mg/kg of body weight by means of a gastric gold tube.

Table 2. Phytochemical screening of secondary metabolites presents in the ethanolic extract of the *J. macrantha* stems.

Fraction	Secondary metabolites	Test	Results	Observations
A	Free amino acids	Ninhidrina	+	Violet coloring
	Flavonoids	Shinoda	+	Reddish yellow coloration
	Phenols and tannins	Ferric chloride	+	Bluish-green coloring
B	Terpenes	Lieberman	+	Pink coloration
C	Alkaloids	Mayer	+	Slight orange precipitate
	Coumarins and lactones	Baljet	+	Slight vinous red coloration
	Terpenes	Lieberman	+	Pink coloration
D	Alkaloids	Mayer	+	Slight orange precipitate
	Catechins	Catechin test	+	Turquoise green coloring to the ultraviolet spectrum
	Coumarins	Baljet	+	Slight vinous red coloration
	Flavonoids	Shinoda	+	Reddish yellow coloration
	Terpenes	Lieberman	+	Pink coloration
E	Catechins	Catechin test	+	Turquoise green coloring to the ultraviolet spectrum
	Flavonoids	Shinoda	+	Reddish yellow coloration
F	Saponins	Foam	+	Foaming that lasts over time

Results legend: presence of metabolite (+); absence of metabolite (-)

Ethical aspects

The study was carried out in strict compliance with international (EU Directive 2010/63/EU for experiments on animals) and national ethical standards on experimental procedures and animal care; and based on the certificate of consent for the handling of animals approved by the Research Ethics Committee of the San Luis Gonzaga National University of Ica, by CEI-UNICA certificate No 001/04-2021.

Statistical analysis

The data obtained through the collection form were entered into a database in the Microsoft Excel program. These databases were exported to IBM's SPSSTM (Statistical Package for the Social Sciences) program for statistical analysis.

Analysis of variance (ANOVA) and Dunnett's multiple comparison test were applied to the results with a $p<0.05$ as statistically significant. Values were reported as mean ± standard deviation.

RESULTS

In Table 2, it can be seen that the ethanolic extract tested positive for a series of secondary metabolites, among which we can highlight the compounds of phenolic types, flavonoids, alkaloids and catechins, determined by phytochemical screening.

Table 3 shows a comparison of the phytochemical screening of the secondary metabolites found in the extracts of leaves, stems and bark of *J. macrantha* according to the area of origin, found in the extracts of the leaves and stems of the aforementioned species that grows in the Pampas River (Tinco et al., 2011) and in the San Francisco de Pujas area of the Vilcashuamán province, Ayacucho department (Aguilar, 2015), secondary metabo-

lites, similar to that observed in the samples collected from the Socco annex, Tibillo district, Palpa province, Ica region, southern Peru. While in the sample collected in the district of Orcopampa, province of Castilla, region of Arequipa, 2016, flavonoids and terpenes were identified, the tests for alkaloids and tannins were negative (Angulo and Jara, 2016).

In a model of 1% histamine-induced bronchospasm induced in guinea pigs, the bronchodilator effect of the ethanolic extract of *J. macrantha* stems was evaluated at a single dose and orally. The latency period that begins with the first cough and the intensity of the effect, observed by asphyxia and anaphylaxis, were used as measurement parameters. The high bronchodilator effect was obtained at a dose of 400 mg/kg. The average values are presented in Table 4.

Fig. 1 shows the comparisons of the latency period of the extracts of *J. macrantha* with the positive control drug and placebo as a negative control, observing that at the dose of 400 mg/kg, the first cough is observed at the 33 s.

Fig. 2 shows comparisons of the intensity of the effect, measured by asphyxia (generated by histamine that occludes the trachea) and anaphylaxis. It is observed that at a dose of 400 mg/kg, asphyxia occurs at 84.5 s and anaphylaxis at 105.6 s, indicating a bronchodilator effect.

Table 3. Secondary metabolites identified from *J. macrantha* extracts according to the area of origin.

Secondary metabolites/ assay	***Jatropha macrantha* Müll.Arg.**			
	Socco, Tibillo Palpa, Ica	River Valley Pampas, Vilcashuamán, Ayacucho 2011 (Tinco et al., 2011)	San Francisco de Pujas, Vilcashuamán, Ayacucho 2015 (Aguilar, 2015)	Orcopampa, Castilla, Arequipa, 2016 (Angulo and Jara, 2016)
Alkaloids	+	+	+	-
Free amino acids	+	+	+	ND
Catechins	+	+	+	ND
Coumarins and lactones	+	+	+	ND
Phenols and tannins	+	+	+	-
Flavonoids	+	+	+	+
Saponins	+	+	+	ND
Terpenes	+	+	+	+

(+): presence of metabolite; (-): absence of metabolite; ND: not determined.

Table 4. Bronchodilator effect of the *J. macrantha* ethanolic extract in single oral administration.

Group	**Drug/placebo**	**Dose (mg/kg)**	**Latency period**		**Effect intensity**	
			First cough (s)	**Number of coughs/ 20 s**	**Asphyxia (s)**	**Anaphylaxis (s)**
G-1	Physiological saline	1 mL/kg	24.5 ± 1.69*	12.0 ± 0.82*	42.5 ± 1.08*	46.5 ± 0.82*
G-2	*J. macrantha* 1 extract	200	29.0 ± 1.34*	4.3 ± 0.96*	54.0 ± 1.23*	73.5 ± 1.57*
G-3	*J. macrantha* 2 extract	400	33.0 ± 1.02*	2.5 ± 0.58*	84.5 ± 1.08*	105.6 ± 0.59*
G-4	*J. macrantha* 3 extract	800	29.0 ± 1.69*	2.3 ± 0.50**	47.0 ± 0.81*	61.5 ± 1.41*
G-5	Chlorphenamine	10	87.0 ± 0.47*	1.0 ± 0.00*	95.2 ± 0.63*	152.3 ± 0.93*

Results are expressed as means ± SD (n = 4), asterisks indicated statistically significant p values compared to the positive control (*p = 0.0001, p<0.05) and not significant (**p = 0.054, p>0.05).

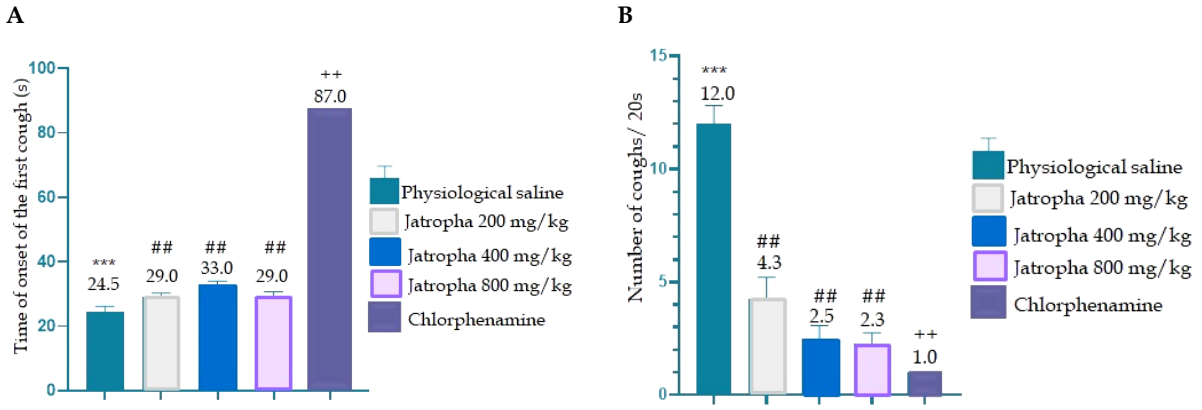

Figure 1. Latency period of the extract of *J. macrantha* measured in the onset of the first cough **(A)** and in the number of coughs in 20 s **(B)**.

The comparison of the effect of the extract of *J. macrantha* at three doses with chlorphenamine, and chlorphenamine with physiological saline, is observed. The data represent the means of the effects (n = 4). The analysis of variance such as Dunnett's multiple comparisons test, present values significantly different from each other ($p<0.05$), $^{++}p<0.0001$ *vs.* negative control ***, $^{\#\#}p = 0.0001$ *vs.* positive control^{++}.

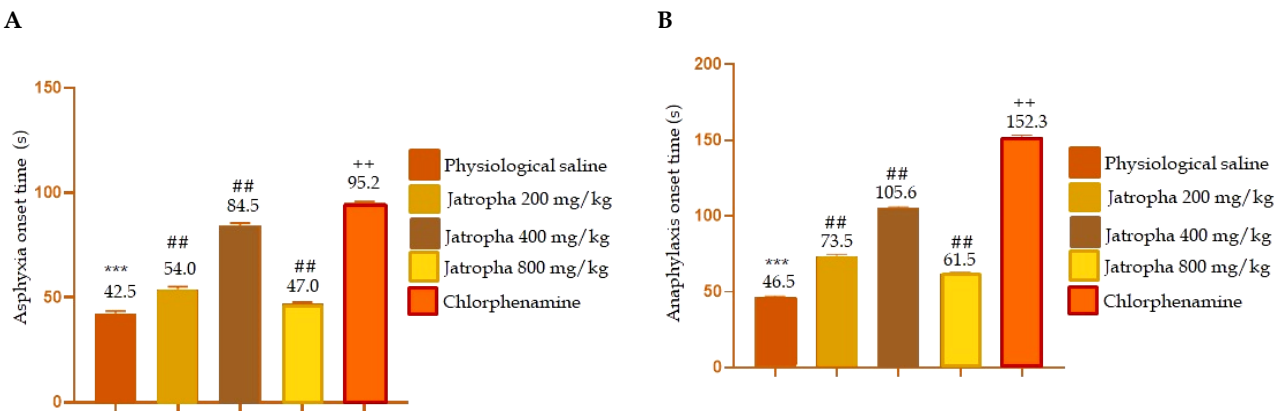

Figure 2. Intensity of the effect of the extract of *J. macrantha* observed by the time of onset of asphyxia **(A)** and anaphylaxis **(B)**.

The means of the effects of *J. macrantha* extract were observed at three doses compared with chlorphenamine, and chlorphenamine with physiological saline. The data represent the means of the effects (n = 4). The analysis of variance such as Dunnett's multiple comparisons test, present values significantly different from each other ($p<0.05$), $^{++}p<0.0001$ *vs.* negative control ***, $^{\#\#}p = 0.0001$ *vs.* positive control^{++}.

DISCUSSION

In the present investigation, the phytochemical screening of the ethanolic extract of *J. macrantha* stems confirmed secondary metabolites such as alkaloids, catechins, coumarins, phenols, flavonoids and tannins. These results are consistent with that reported by previous studies, such as that of Tinco et al. (2011), who collected their plant sample in the Pampas River valley, Vilcashuamán, Ayacucho, and that of Aguilar (2015), who sampled the same plant species in the community of San Francisco de Pujas, Vilcashuamán, Ayacucho. While Angulo and Jara (2016) collected the same species in the area of Orcopampa, Castilla, Arequipa, finding the presence of flavonoids and terpenes. Benavides et al. (2006) isolated catechin-7-O-β-glucopyranoside and proanthocyanidin β-3 from the methanolic extract of the stems of *J. macrantha* (huanarpo). Heredia et al. (2016) reported that by mixing cyclohexane-ethanol solvents, a concentration of 2.15% of total alkaloids expressed as tropenic alkaloids are obtained. Regarding its

popular use, Pardo (2002) described that the macerates of the stems of *J. macrantha* are used in asthma. This traditional use being described by Apaza et al. (2020) in recent years, so it was necessary to do experimental studies to validate the traditional use. In this sense, we have shown that the ethanolic extract of *J. macrantha* stems prolong the appearance of the first cough of the latency period and the frequency of cough in 20 s. At the same time, it prolongs the time of onset of asphyxia and histaminergic anaphylaxis, which are the parameters of the intensity of the pharmacological effect.

Observing the bronchodilator effect at the three proposed doses (200, 400 and 800 mg/kg), being higher than the 400 mg/kg dose, and a single oral administration. In previously carried out studies, such as the one reported by De la Cruz (2019), the bronchodilator activity of *J. macrantha* "male huanarpo" was demonstrated, mentioning that said activity is due to the flavonoids isolated from the leaves and stems of the plant. While Aguilar (2015) states that proanthocyanidins (condensed tannins of flavanols-3 polymer type that are metabolized into anthocyanins), extracted from *J. macrantha*, have anti-inflammatory properties, and in another study, it is indicated that they have vasodilatory properties that can correct erectile dysfunctions (Benavides et al., 2006). Also, recently Apaza et al. (2020) have shown that pomolic and euscaphic acids obtained from *J. macrantha* are potential inhibitors of NF-κB production and HIF-1α in tumor cells. It is known that pro-inflammatory and immunological molecules are the molecular bases of asthma bronchospasm, for which the secondary metabolites mentioned could possibly be involved in the relaxation of the bronchial smooth muscle and the inhibition of pro-inflammatory molecules, supporting its bronchodilator effect, which should be corroborated in future studies.

In a previous study carried out by Zarate et al. (1999), it is described that the extracts of *Werneria apiculata* and *Werneria marcida* protect from histaminergic shock for a time of 86-97 s, indicating that this effect is due to the presence of alkaloids and flavonoids. Although it is a different species from ours in this study, it allows us to compare it in terms of the experimental model and the bronchodilator effect. Later Aguilar (2015) showed that the methanolic extract of the leaves and stems of *J macrantha* "male huanarpo" has a bronchodilator effect, at a dose of 300 mg/kg, with 83.65% efficiency. While the most effective dose in our study was 400 mg/kg, which may indicate that the doses would be between 300-400 mg/kg.

The proanthocyanidins of *J. macrantha*, which are also found in fruits, vegetables, cereals, legumes, tea, cocoa, red wine (Benavides et al., 2006), in purple corn (anthocyanins, flavonoids and polyphenols), have antioxidant properties, for the ability to complex with metal ions and macromolecules (Ramos-Escudero et al., 2012); so, this species studied is a promising plant as an antioxidant, which deserves to be studied. Later, Tinco et al. (2011) reported that the methanolic extract of *J. macrantha* has a vasorelaxant effect on the corpus cavernosum of the rat penis. This effect could be attributed to the presence of four flavonoids, such as 6-hydroxy-4', 5, 7-trimethoxy flavone, 4', 7-dihydroxy-5,6-dimethoxyflavone, 7-hydroxy-3', 4', 5', 5,8-pentamethoxyflavone and 4', 7-dihydroxy-3', 5,6–trimethoxyflavone.

The limitations of this study are in the size of the samples of experimental animals (n = 20), not having extracted the secondary metabolites and not having elucidated the chemical structure of the metabolites responsible for the molecular mechanism of action and therefore their activity bronchodilator, which is being considered by our research group, to be evaluated in future studies. Notwithstanding the foregoing, we consider this study to be relevant, as it contributes to rescuing and enhancing the popular use of this medicinal plant and making scientific evidence about its bioactive properties, which will encourage researchers to elucidate the chemical structures of metabolites, establish the chemical structure/activity relationship and the molecular mechanism of action of bronchodilator activity, so that it is of interest in clinical practice.

CONCLUSIONS

The phytochemical study of the ethanolic extract of *J. macrantha* indicates secondary metabolites such as alkaloids, catechins, coumarins, phenols, flavonoids and tannins, which would be responsible for the bronchodilator effect, at a medium effective dose 400 mg/kg, orally, in *Cavia porcellus*. Scientific evidence suggests further research to quantify and elucidate the chemical structure responsible for the activity and, thus, establish it as a therapeutic alternative.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Chávez H	Palomino F	Angelino J	Torres E	Bendezú MR	García JA	Loja B	Muñoz AM	Alvarado AT
Concepts or ideas	x	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x			x
Definition of intellectual content					x	x	x	x	x
Literature search	x	x	x	x					
Experimental studies	x	x	x	x	x	x	x	x	x
Data acquisition		x	x				x		
Data analysis	x	x	x				x		
Statistical analysis							x		
Manuscript preparation	x			x	x	x		x	x
Manuscript editing	x	x	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x	x	x

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