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Pharmacological and Toxicological Study of a Traditional Mayan Herbal Preparation Used as Antihypertensive Agent

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ASR and VYP designed the ex vivo and hypotensive study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HARZ and ROA designed the toxicology study and histopathological analysis, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MIB and GÁV wrote the protocol and wrote the first draft of the manuscript. Author SFG identified vegetal species. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To evaluate preparation herbal mixed of *Pouteria campechiana*, *Chrysophyllum cainito*, *Citrus limonum* and *Annona muricata* (PCCA) on vasorelaxant and hypotensive effect on rat model and toxicological data after acute oral administration to give scientific support to the use ethnomedical and to explore their potential damaging on oral intake.
Study Design: Experimental.
Place and Duration of Study: Sample female and male Wistar rats. Pharmacology laboratory of Chemistry School and Department of Clinical and Epidemiological Research of Medicine School, Autonomous University of Yucatán. Between October 2014 and July 2016.
Methodology: An ethanolic extract of PCCA was prepared at a ratio of 1:1:1:1 of each plant plus individual extracts were prepared. Vasorelaxant effect was assessed (3.03 to 100 µg/mL), hypotensive effect ((100, 200, 300 mg/Kg) and median lethal dose (LD_{50}) by oral acute toxicity method (OECD 423 guide).
Results: PCCA extract induced a significant vasorelaxation (medium effective concentration (EC_{50})=463.43 µg/mL) in a concentration-dependent manner in aorta's endothelium-intact rings and this effect was partially endothelium-dependent. Acute oral administration of 200 and 300 mg/kg of PCCA exhibited significant decrease in systolic blood pressure in normotensive rats. PCCA did not show clinical toxicity of acute oral administration. Only 2000 mg/kg show histopathological inflammatory responses on gut and liver.
Conclusion: PCCA induces a significant cardiovascular effect and was not toxic for rodents. The results support the popular use of some Mayan Medicinal plants as antihypertensive agents; however, clinical studies are necessary.

Keywords: Acute toxicity; Annona muricata; Citrus limonum; Chrysophyllum cainito; hypotensive effect; Pouteria campechiana; traditional Mayan medicine.

1. INTRODUCTION

Nowadays, in some parts of the world, many health problems are prevalent in the population despite the drugs used to treat them; people commonly use alternative therapies such as medicinal plants. Likewise, the World Health Organization (WHO) encourages developing countries to study popular medicine to contribute in the treatment of common diseases [1]. Hypertension is one of the non-transmissible diseases with greater impact worldwide [2]. In addition, it is a major risk factor for other diseases like stroke, acute myocardial infarction and renal failure [3]. It has been estimated that 9.4 million annual deaths worldwide are due to hypertension [1] and Mexico is one of the countries with higher prevalence with approximately 31.6% of its population suffering from this disease [4]. The treatment has as purpose the stabilization of arterial tension to optimal levels [5] and even though low-cost therapy is available, many rural communities do not have access to it and draw on herbal preparations [6].

Mexican southeast is where Mayan culture was located. It is known for having an extensive knowledge of nature and how to use it as popular medicine. Despite the passing of centuries, this region still preserves the tradition of using medicinal plants for the treatment of diseases. In Yaxcabá, Yucatán, a herbal preparation (PCCA) is consumed with a mixture of 1:1:1:1 ratio of leaves of *Pouteria campechiana* (Kunth) Baehni*, Citrus limonum* (L.) Osbeck*, Chrysophyllum cainito* L. and *Annona muricata* L. This mixture is usually used for the treatment of diabetes, pain and hypertension [7-11] and there are reports of pharmacological activities such as antinociceptive and antihyperalgesic effects [8]. However, there are no studies supporting the cardiovascular effect or toxicological evidence of oral consumption of PCCA. In this matter, we decided to evaluate the herbal preparation PCCA used by Mayan communities on vasorelaxant and hypotensive effects and also, toxicological data on acute oral administration was obtained to give scientific support to its therapeutic use and to explore its damaging potential on oral intake.

2. MATERIALS AND METHODS

2.1 Chemicals

Carbamoylcholine chloride 99% (carbachol), Diltiazem hydrochloride, Norepinephrine L-bitartrate hydrate 99% (NE) were purchased from

Sigma-Aldrich Co (St. Louis, MO, USA). All others reagents were analytical grade from local sources. Stock solutions of the extracts were dissolved with dimethyl sulfoxide 1% (DMSO) and freshly prepared the same day of experimentation.

2.2 Plant Material and Preparation of Single Extracts and PCCA Extract

Aerial parts of *Annona muricata* L. (*Annonaceae*), *Citrus limonum* (L.) Osbeck. (*Rutaceae*), *Chrysophyllum cainito* L. (*Sapotaceae*) and *Pouteria campechiana* (Kunth) Baehni. (*Sapotaceae*) were collected in October 2014 in Mérida, Yucatán, México. They were collected by Rolffy Ortiz-Andrade, Ph.D. and identified by Salvador Flores-Guido, Ph.D. Specimens of each plant were deposited on "Alfredo Barrera Marín" herbarium at Facultad de Medicina Veterinaria y Zootecnia of Universidad Autonóma de Yucatán (UADY). The details of medicinal plants with their acquisition code numbers (voucher) are listed in Table 1.

The air-dried leaves of the plants were combined to obtain the ethanol extract. A 2 Kg mixture of the four plant species (rate 1:1:1:1, 500 g each) was ground into a powder (mesh 2 mm) and extracted with ethanol by infusion for 90 min. After filtration, the organic extract (PCCA) was obtained under reduced pressure. The rate and extraction conditions selected for mixture preparation were provided by a traditional Mayan healer ("H-men") of the Regional Council of Indigenous Doctors (Consejo Regional de Médicos Indígenas) NACHI-COCOM, A.C., Mérida, Yucatán. In addition, individual extracts of each species were prepared following the same methodology. Single extracts and PCCA were lyophilized (Freezone 6, Labconco®) to assure maximum dryness and percentage yields were calculated (Table 2).

2.3 Animals

Male Wistar rats (250-300 g) were used *in ex vivo* and *in vivo* evaluation. Nulliparous female Wistar rats (250-300 g) were used for toxicological studies. Centro de Investigaciones Regionales "Dr. Hideyo Noguchi", from Universidad Autonóma de Yucatán, supplied both. Experimentation animals were maintained under standard laboratory conditions (12 h light/dark cycle, 25 ± 2ºC and 45-65% of relative humidity) with free access to food and water. All animal procedures were conducted in accordance with the Mexican Federal Regulations for Animal Experimentation and Care [12] and were approved by the Internal Animal Care and Use Committee.

2.4 *Ex vivo* Pharmacological Evaluation

2.4.1 General conditions

The rats were sacrificed by cervical dislocation after deep anesthesia using ether; thoracic aorta was removed and cleaned from adhering connective tissue. Aorta was cut into 3-5 mm length rings. Aortic rings were mounted using stainless steel hooks, under an optimal tension of 3 g for 60 min in 10 mL organ baths containing warmed (37ºC) and well-oxygenated (O_2:CO_2, 95:5%) Krebs solution (composition, mM: NaCl 119, KCl 4.6, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.5, $NaHCO_3$ 20, glucose 11.4 and EDTA 0.027). Changes in isometric tension were recorded by Grass-FT03 force transducers (Astromed, West Warwick, RI, USA), connected to a MP150 analyzer (BIOPAC Instruments, Santa Bárbara, CA, USA), as previously described [13]. After stabilization, rings were contracted by NE [1 μM],

Table 1. Botanical and common names of plant species contained in PCCA extract, specimen identification voucher and traditional medicinal uses

Plant name (Family)	Common name	Voucher number	Medicinal use
Annona muricata L. (*Annonaceae*)	Guanábana, Tak' ob, Tak' op, Tak' oop	12870	Bronchitis, cough, asthma, hyperlipidemia, diabetes, hypertension [7]
Chrysophyllum cainito L. (*Sapotaceae*)	Caimito, Cayumito	12871	Diarrhea, obesity, diabetes, hypertension [7]
Citrus limonum (L.) Osbeck (*Rutaceae*)	Limón	12868	Diabetes, hypertension [7,9,11]
Pouteria campechiana (Kunth) Baehni (*Sapotaceae*)	Canistel, Ocotillo, Mante, Kanicte'	12867	Fever, as antiseptic, as somniferous, hypertension [9-11]

after reaching maximum contraction the rings were washed with Krebs solution; this procedure was performed by triplicate. The absence of endothelium was confirmed by the lack of a relaxing response to carbachol [0.1 µM].

Table 2. Yield of extraction process of PCCA extract and single species extracts

Sample	Extraction yield (%)
PCCA extract	5.19
A. muricata	4.65
C. cainito	5.10
C. limonum	12.86
P. campechiana	10.89

2.4.2 Vasorelaxant activity

After equilibration, each aorta ring was pre-contracted with NE. When contraction reached a plateau, single extracts or PCCA extract (3.03 to 1000 µg/mL), vehicle (1% of DMSO; maximum concentration) and positive controls (carbachol: $6.28X10^{-4}$ to 1.83 µg/mL and nifedipine: $3.89x10^{-5}$ to 3.46 µg/mL) were added to the bath in cumulative concentrations. Then, concentration-response curves were obtained (n=5). The vasorelaxant effect of extracts and positive controls were determined by comparing the muscular tone of the contraction before and after the application of the test materials. Muscular tone was calculated from the tracings, using Acknowledge 4.1 software (Biopac System, Inc., Santa Barbara, CA, USA).

2.5 *In vivo* Hypotensive Evaluation

Hypotensive activity of PCCA extract evaluation was conducted in conscious normotensive adult rats. They were randomly allotted into five groups of five animals each (n=5): [group 1 (vehicle, saline solution (SS), 5 mL/Kg), group 2 (diltiazem, 30 mg/Kg, as positive control), group 3 (PCCA extract, 100 mg/Kg), group 4 (PCCA extract, 200 mg/Kg) and group 5 (PCCA extract, 300 mg/Kg)] all were given a single intragastric dose. Measurements (blood pressure and heart rate) were recorded before and after the treatment of each group at 0, 1, 3, 5 and 7 h by a tail cuff method using a LE 5001 automatic blood pressure computer (PanLab®, Harvard Apparatus, Spain). Percent decrease in heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were calculated.

2.6 Acute Toxicological Studies

Modified validated protocol of OECD test guidelines was utilized [14]. Female rats were allotted into five groups (n=3): control group (SS, 5 mL/Kg) and four groups treated with PCCA extract (5, 50, 300, 2000 mg/Kg). Each group was administered by I.G. route and observed four hours after administration (considered as day one). Afterwards, body weight was monitored weekly. The rats were observed daily for abnormality signs during 14 days. At the end of treatment, the rats were sacrificed by cervical dislocation after deep anesthesia using ether. Blood samples were obtained by direct cardiac puncture for biochemical analysis and kidney, liver and gut were obtained for histopathological analysis.

2.6.1 Blood biochemical profile determination

For biochemical analysis, blood samples were obtained in Vacutainer® tubes with EDTA for hematic cytometry and tubes without anticoagulant for glucose, cholesterol, triglycerides and hepatic enzymes determination. Samples in tubes without anticoagulant were centrifuged at 3500 rpm during 10 min to obtain serum, which was stored at -18°C. Levels of leukocytes, erythrocytes, platelets, hemoglobin and hematocrit were measured with a Sysmex pocH-100i autoanalyzer. Glucose values were obtained by enzymatic colorimetric test GOD-POD, CHOD-POD for cholesterol and GPO-POD for triglycerides using kits (Spinreact®). Glutamic oxaloacetic transaminase (GOT or AST) and Glutamic pyruvic transaminase (GPT or ALT) were measured using the NADH kinetic UV test (Wiener lab®). All measurements were performed in Erba Mannheim® Chem-7 UV-Vis spectrophotometer.

2.6.2 Histopathological analysis

Briefly, animals (n=3) for each condition were sacrificed under deep anesthesia using ether and liver, kidney and gut were removed and were fixed with 100 mL of 10% formaldehyde solution for 3 days. Then the tissues were processed according to standard paraffin technique. Cross-sections of 5-micron thickness were stained with hematoxylin/eosin. All images were analyzed by an expert with blind technique and captured with Niko Eclipse E2000 microscope.

2.7 Data Analysis

Results of vasorelaxant and hypotensive effects were expressed as the mean ± S.E.M of five experiments (n=5) and results of acute toxicity

were expressed as the mean ± S.E.M of three animals per group (n=3). Concentration-response curves (CRC) were plotted, and experimental data from the CRC were fitted to the nonlinear (Hill-equation) curve-fitting program (ORIGIN PRO 10). Statistical significance of differences between means was assessed by a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. *P*-values less than 0.05 (*P =0.05) were considered to be statically significant [15,16].

3. RESULTS AND DISCUSSION

3.1 Vasorelaxant Effect

Vasorelaxant effect of PCCA extract and single plants were examined, Fig. 1 shows the relaxation CRC of PCCA extract and controls. PCCA extract induced a significant vasorelaxation [maximum effect (E_{max})=65.78 ± 2.60%, Medium effective concentration (EC_{50})=463.43 µg/mL] in a concentration-dependent manner in endothelium-intact aortic rings (E+) and this effect was partially endothelium-dependent [Endothelium-denuded rings (E-); E_{max}=42.21 ± 4.06%, significance *p<0.05].

Single plant extracts were evaluated to find which species were responsible of the effect showed by PCCA extract. Fig. 2A shows the effect of the single species. *A. muricata* (E+; E_{max}=59.9 ± 8.34%) and *Citrus limonum* (E+, E_{max}=61.09 ± 8.22%) consisting of a concentration-dependent and partially endothelium-dependent vasorelaxant effect (Fig. 2B). *C. cainito* (E_{max}=34.57 ± 6.69%) induced moderate vasorelaxation of E+ rings, but did not have any effect in E- rings (Fig. 2C). *P. campechiana*, showed a biphasic effect (Fig. 2D), at low concentrations (<100 µg/mL) it produced significant vasorelaxation (E_{max}=59.21 ± 4.75%) and at higher concentrations it produced vasoconstriction (E_{max}=12.75%). This biphasic-effect is only seen in endothelium-intact aortic rings (E+) (Fig. 2D). PCCA extract and single species were not more efficient or potent than control drugs [carbachol (rings E+) and nifedipine (rings E-)].

3.2 Hypotensive Effect

Oral administration of 200 mg/Kg of PCCA extract showed a significant decrease in systolic blood pressure at 1h (12%; p=0.008), 3h (12.5%; p=0.001) and 5h (10%; p=0.001) after administration (Fig. 3A). In a similar way, oral administration of 300 mg/Kg of PCCA extract showed significant decrease in systolic blood pressure at 1h (5.77%; p=0.035), 3h (9.19%; p=0.011) and 5h (10%; p=0.011) after administration. Both doses caused blood pressure to return to baseline after seven hours (7h). 100 mg/Kg of PCCA extract did not cause significant decrease in systolic blood pressure. The diastolic blood pressure and heart rate were unmodified compared to control (SS) (Fig. 3B and 3C).

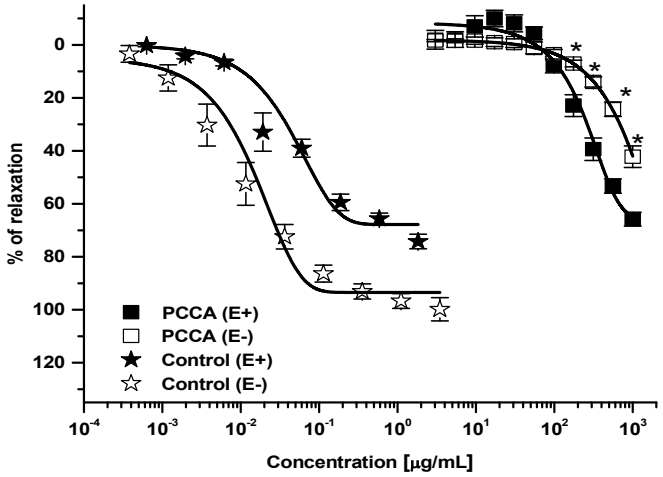

Fig. 1. Concentration-response curve (CRC) of PCCA. Results are expressed as the means ± SEM of five experiments. (*p< 0.05, comparison between rings with endothelium and without endothelium)

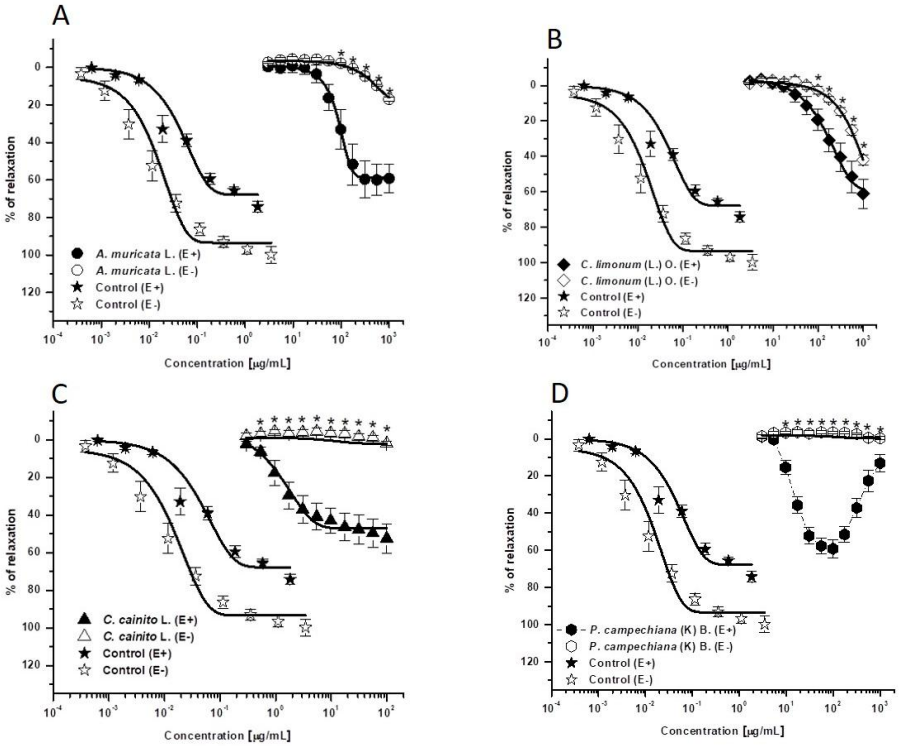

Fig. 2. Concentration-response curve (CRC) of A) *A. muricata* L. B) *C. limonum* (L.) Osbeck C) *C. cainito* L., D) *P. campechiana* (Kunth) Baehni extracts. Results are expressed as the means ± SEM of five experiments. (*) Indicates significance on comparison between rings with endothelium and without endothelium (p< 0.05)

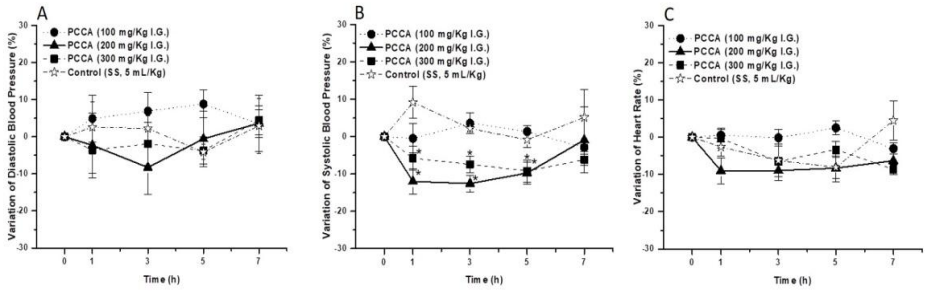

Fig. 3. Maximal decrease in A) systolic blood pressure, B) diastolic blood pressure and C) heart rate elicited by oral administration of 100, 200 and 300 mg/Kg of PCCA in conscious rats. Results are expressed as the means ± SEM of five rats per group. (*) Indicates significance respect to saline solution (SS) (P<0.05)

3.3 Toxicological Data

In order to establish the potential damage and likely toxicological effects caused by acute oral administration of PCCA extract, independent groups of rats were administrated once with 5, 50, 300, or 2000 mg/Kg of PCCA extract and then observed during 14 days. No rats exhibited any visible signs of toxicity as tremors, convulsions, salivation, diarrhea, lethargy, sleep, piloerection nor physiological changes in skin and fur, eyes or mucous membranes. PCCA extract did not affect locomotor activity or food consumption. No deaths were observed in the treated rodents.

The results of blood biochemical analysis were normal without difference in levels of erythrocytes, platelets, hemoglobin, hematocrit, glucose, cholesterol, GOT and GPT. In contrast, levels of leukocytes (at 5 mg/Kg) and serum levels of triglycerides (at 2000 mg/Kg) were significantly increased (Data not shown).

3.4 Histological Findings

14 days after the administration of PCCA different doses, liver, kidney and large intestine were analyzed by histopathology. No morphological differences were found between control animal tissues (which were administrated saline solution) and experimental animals (administrated 50, 100 or 300 mg/Kg of PCCA extract). Animals that were administrated 2000 mg/Kg of PCCA extract showed increased inflammatory cells in the liver, as well as increased sinusoidal spaces. Fig. 4. The intestine presented a slight increase of epithelial mucous cells as well as inflammatory infiltration in the subepithelial tissue. We did not find pathological changes in the kidney, more details in Table 3.

4. DISCUSSION

In Yucatán peninsula, many medicinal plants are used either alone or in mixtures of two or more plants as treatment of chronic degenerative diseases as hypertension or diabetes among others [7,9-11]. PCCA mixture is used day-to-day in Yaxcabá, a rural community of Yucatán México as antihypertensive agent [9-11]; this work shows scientific evidence of pharmacological and toxicological effects of Mayan medicinal potion (PCCA extract) and plants contained in it.

PCCA extract showed vasorelaxant effect in both endothelium-intact and endothelium-denuded aortic rings. This suggests that the vasorelaxant effect is partially endothelium-dependent [17] and may be caused by endothelium-derived relaxing factors (EDRF) [18] and/or by factors produced directly in vascular smooth muscle cells (VSMC) [19]. Nitric oxide (NO) is the most important EDRF. NO is produced in endothelial cells and diffuses into VSMC where it activates soluble guanylate cyclase (sGC), which produces cyclic guanosine monophosphate (cGMP); this cyclic nucleotide performs several cellular functions by activating cGMP-dependent protein kinases (PKG) that result in relaxation [20]. Another EDRF are prostaglandins, which are produced when calcium activates phospholipase A_2, which liberates arachidonic acid that is then converted to prostaglandin I2 (PGI_2) to induce vasorelaxation [21]. Other endothelium-derived vasoactive mediator that could cause the vasorelaxant effect is the endothelium-derived hyperpolarizing factor (EDHF), which causes vasodilation by hyperpolarizing vascular smooth muscle cells through the stimulation of potassium ion channels to permit this ion's efflux, leading

Table 3. Histological findings of tissue obtained of rodents administered with different doses of PCCA extract

Treatment I.G	Tissue	Morphological damage			
		Necrosis	Inflammatory infiltrate	Cellular swelling	Fat infiltration
Control (SS,5 mL/Kg)	Liver	No	No	No	No
	Kidney	No	No	No	No
	Gut	No	No	No	No
PCCA 5 mg/Kg	Liver	No	No	No	No
	Kidney	No	No	No	No
	Gut	No	No	No	No
PCCA 50 mg/Kg	Liver	No	No	No	No
	Kidney	No	No	No	No
	Gut	No	No	No	No
PCCA 300 mg/Kg	Liver	No	No	No	No
	Kidney	No	No	No	No
	Gut	No	No	No	No
PCCA 2000 mg/Kg	Liver	No	++	+	No
	Kidney	No	No	No	No
	Gut	No	+	No	No

+, ++, +++ Represent qualitatively the degree of the characteristics evaluated; PCCA: extract herbal; SS: Saline solution

to the inhibition of calcium influx to the cell via voltage-dependent calcium channels (VDCC) [22].

Due the unspecific mechanism of action, we can't discard the possible simultaneous participation of endothelium-independent relaxation mechanisms acting directly on VSMC such as VDCC blockade, antagonism of α_1, α_2 and/or β adrenergic receptors, intracellular calcium recapture by sarcoplasmic reticulum (SR) or activation of calcium-activated potassium channels ($BKCa^{2+}$) through protein kinase G (PKG) [23] among others.

In the best of our knowledge, four medicinal plants, *A. muricata*, *C. limonum*, *C. cainito* and *P. campechiana* compose the PCCA extract. In order to determine the effect of single species, we assessed their vasorelaxant effect.

A. muricata and *C. limonum* effects were partially endothelium-dependent so they may involve all the mechanisms described previously for PCCA extract. *C. cainito* showed less effect than *A. muricata* and *C. limonum* and their effect was completely endothelium-dependent. On this basis, their mechanism of vasorelaxation could only involve EDRF described previously [18]. *P. campechiana* showed a biphasic effect and was completely endothelium-dependent since it produced a vasorelaxant effect at low concentrations, but a contractile effect at higher concentrations. This behavior suggests the presence of metabolites with dual effect on vascular tension [24] or with opposite effects and different potency. At a low concentration the effect of the most potent metabolites is observed (vasorelaxant effect) while at a higher concentration the effect of less potent or opposite effect metabolites (less vasorelaxation or vasoconstrictor effect, respectively) is observed [25]. Vasorelaxant effect showed by *P. campechiana* is completely endothelium-dependent; this gave us evidence that the possible vasorelaxant mechanism of action could be through the participation of EDRF. Contractile effect could be caused by contractile factors inherent to vascular endothelium such as production of endothelin isoform ET-1 causing the stimulation of phospholipase C (PLC), formation of inositol 1, 4, 5-triphosphate (IP_3) and calcium release from SR [19].

Based on this study, *C. limonum, A. muricata and P. campechiana* species may be responsible of the PCCA extract vasorelaxant effect. Thus, our results of *ex vivo* vasoconstrictor evaluations may give evidence of possible side effects in human oral consumption of *P. campechiana*. It is very important to know effective concentrations of medicinal plants because at high doses severe side effects might appear.

In the *in vivo* assay, PCCA extract only showed significant systolic blood pressure decrease at 200 and 300 mg/Kg doses. This effect could be associated to vasorelaxant ability, since at low concentrations, there was slight *ex vivo* contraction and at higher concentrations it caused efficient vasorelaxant effect. Previous pharmacological studies carried out with the individual species contained in our PCCA extract showed the hypotensive activity of *A. muricata* [26]. This suggests that PCCA extract *in vivo* effect on systolic blood pressure may be attributed to the presence of *A. muricata.*

Oral acute administration of PCCA extract did not exhibit toxicity signs or deaths at any of the doses administrated (<2000 mg/Kg). This suggests PCCA may be catalogued as category 5 substances according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and set lethal doses above 2000 mg/Kg [14]. Blood biochemical profiles were unmodified, since no altered levels of transaminases; neither glucose concentrations were found, suggesting that sample tests did not produce hepatic toxicity. High values of leukocytes and triglycerides at 5 mg/Kg were found but cannot be attributed to PCCA extract administration since we did not have control of food intake amount (which could elevate triglycerides levels), neither infections in the study subjects (that could increment leukocytes levels). Histopathological analysis showed inflammatory cells increase at the highest dose suggesting that PCCA extract might cause chronic oral toxicity in high doses (Fig. 4). In the upper row representative images of the hepatic tissue are shown in the different treatment conditions (Fig. 4A-E), in Fig. 4E, showing inflammatory cells (arrows). Dilation of the sinusoidal spaces, since doses of 50 mg/Kg and increases with the doses.

In the middle row, images of the intestinal mucosa (Fig. 4F-J) are shown, since the other layers of the intestine showed no morphological changes (data not shown). It is observed that from the dose of 50 mg / kg there is an increase in goblet cells (arrows) as well as inflammatory

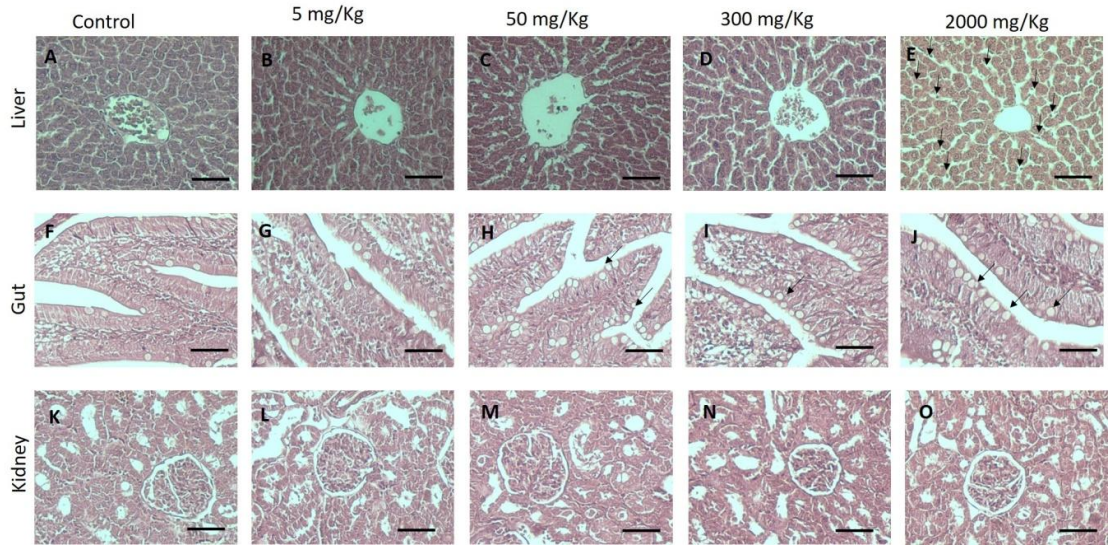

Fig. 4. Histopathological analysis. The analysis was performed with blinded technique, in triplicate each treatment condition calibration bar 50 microns

infiltrate in the loose connective tissue of the intestinal villi.

Images of renal tissue are shown on the bottom line (Fig. 4K-O). No morphological differences were found between the control and the different treatments.

5. CONCLUSION

In conclusion, PCCA extract induces hypotensive and vasorelaxant effect in a dose and concentration-dependent manner, respectively. It was not toxic in oral acute administration of rodents. These results help support the popular use of some botanical species in the PCCA extract as hypotensive agent; however, clinical studies will be required to confirm their efficacy in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animal procedures were conducted in accordance with the Mexican Federal Regulations for Animal Experimentation and Care [12] and were approved by the Chemistry School Animal Care and Use Committee and approved by the Institutional Animal Care and Use Committee based on US National Institute of Health publication [27].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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